Cell. Mol. Life Sci. (2012) 69:783–791
DOI 10.1007/s00018-011-0777-4

Cellular and Molecular Life Sciences

RESEARCH ARTICLE

Monitoring *mmp-9* gene expression in stromal cells using a novel transgenic mouse model

Katherine Biron-Pain · Yves St-Pierre

Received: 15 May 2011 / Revised: 27 June 2011 / Accepted: 19 July 2011 / Published online: 11 August 2011

Abstract Matrix metalloproteinase (MMP)-9 (gelatinase B) is involved in extracellular matrix degradation in the context of the motility and in in vivo migration of normal and malignant cells. Accordingly, its expression is highly regulated at the transcriptional level. In several types of human cancers, MMP-9 expression is abnormally elevated and has been associated with poor prognosis. Such high levels of MMP-9 expression are found in tumor cells and in stromal components. Therefore, it is important to understand the spatiotemporal expression pattern of MMP-9 in tissues for the development of effective therapeutic strategies that are aimed at suppressing *mmp-9* gene activation. In the present work, we describe a transgenic mouse model harboring a *luciferase* gene under the control of the murine *mmp-9* promoter. We found that the expression pattern of the transgene was similar to that of the endogenous *mmp-9* gene either constitutively or following inflammatory stimuli. A constitutive transgene expression was observed in the bone marrow, consistent with the observed high levels of endogenous *mmp-9* gene expression normally found in the bone. LPS injection in mice also induced a consistent and significant increase in bioluminescent signals in the liver, which is a major target of LPS-induced septic shock. Finally, we further used the model to provide evidence that *mmp*-9 is activated in stromal cells of the lung and spleen in melanoma tumor-bearing mice. This bioluminescent imaging model may facilitate in vivo monitoring of MMP-9 activation in stromal cells in tumor progression and inflammatory diseases.

K. Biron-Pain · Y. St-Pierre (✉)
INRS-Institut Armand-Frappier, 531 Boul. Des Prairies, Laval, QC H7V 1B7, Canada
e-mail: yves.st-pierre@iaf.inrs.ca

Keywords *mmp*-9 · Transgenic cancer · Melanoma · Metastasis

Introduction

Members of the matrix metalloproteinase (MMP) family encode zinc-dependent endopeptidases that play an important role in the turnover of extracellular matrix (ECM) in physiological and pathological processes. The expression of MMPs is highly regulated, and their expression is limited under quiescent conditions such as in normal mature tissues. In cancer cells, however, MMPs are constitutively expressed at high levels and play an essential role in tumor progression, invasion and metastasis formation [1]. Although MMPs represent important therapeutic and diagnostic targets for the treatment and detection of human cancers, the development of therapeutic strategies that are aimed at inhibiting their functions in cancer has met with little success [2, 3]. A large part of this failure has been attributed to our limited knowledge of their function and expression patterns. Several studies have established that tumor cells express abnormally high levels of MMPs. However, there is increasing evidence that their expression is not restricted to the tumor cells themselves but is also found in stromal cells that constitute the tumor microenvironment.

The importance of stromal-derived MMPs in cancer has been examined in several tumor models, which have shown that stroma-derived MMPs are an important contributor to different stages of tumor progression. For example, MMP-9 from bone marrow-derived cells contributed to the tumor incidence of skin carcinoma [4]. In a model of pancreatic islet tumorigenesis, stromal-derived MMP-9 promoted the angiogenic switch [5]. In melanoma models, the lack of

MMP-9 expression by stromal cells impaired the ability of melanoma cells to metastasize [6, 7]. MMP-9 secretion by stromal cells, most notably inflammatory cells, can promote angiogenesis by promoting the release of VEGF [5]. Because MMP-9 is secreted by tumor and stromal cells, assessing its spatiotemporal expression pattern in a given tissue remains an important obstacle to the development of effective therapeutic strategies that are aimed at suppressing the *mmp-9* gene activation in stromal cells. In the present work, we describe a transgenic (Tg) mouse model that harbors the murine *mmp-9* promoter that controls the expression of the firefly *luciferase* gene.

Materials and methods

Generation and analysis of Tg mice

The pGL3-proMMP-9 reporter vector encoding the essential DNA binding motifs for *mmp-9* gene expression was digested using MluI and SalI. A 2.7-kb DNA fragment containing the *mmp-9* promoter and the *firefly luciferase* reporter gene was purified from agarose gel using a standard technique. The purified DNA fragment was then microinjected into the C57BL/6xC3H pronuclei of fertilized mouse oocytes at McGill University via the Quebec Transgenic Research Network (QTRN). After injection, the embryos were transferred to pseudo-pregnant females. At 2–3 weeks of age, pups were ear-tagged, and the tail DNA was purified for transgene detection. Animals were genotyped by PCR using tail DNA and primers that hybridized to the *mmp-9* promoter sequence and the luciferase reporter gene cDNA (5′-AGGAAGGATAGTGCTAGCCTGAGAA GGATG-3′ (sense) and 5′-CTTTATGTTTTTGGCGTCT TCCA-3′ (antisense), respectively. The 5′-CGGAGTCAA CGGATTTGGTCGTAT-3′ (sense) and 5′-AGCCTTCTC CATGGTGGTGAAGAC-3′ (antisense) primers were used for the amplification of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene as a control. The amplification was performed in a PTC-100 thermal cycler (MJ Research, Waltham, MA, USA) using the following protocol: 120 s at 94°C and then 40 cycles of three steps consisting of 60 s at 94°C, 60 s at 64°C and 60 s at 72°C. The reaction mixture was size-separated on an agarose gel. Specifically amplified products were detected using SyBr Safe staining and ultraviolet transillumination. For the primary screening of Tg lines, groups of 9–10 mice including both genders were imaged before the injection ($T = 0$) of lipopolysaccharide (LPS) (0127:B8; Sigma-Aldrich) at 50 mg/kg body weight. The criteria used for screening were as follows: (1) high basal luciferase activity in organs expressing high levels of endogenous MMP-9 mRNA and (2) up-regulation of the luciferase expression in multiple organs following LPS injection. Seven lines were screened, and one line with the best characteristics was selected for characterization. To avoid the background of a hybrid genotype, selected mice were backcrossed to a C57BL/6 strain for at least five generations. Breeder pairs for the C57BL/6 mouse colony were purchased from the Jackson Laboratory. All animals were housed in a specific pathogen-free environment in accordance with the institutional guidelines for animal care. Experiments were performed in accordance with protocols approved by the Institut National de la Recherche Scientifique Institutional Animal Care and Use Committee.

In vivo imaging

Mice were injected intraperitoneally (i.p.) with 150 mg/kg of D-luciferin potassium salt (Regis Technologies, Morton Grove, IL, USA). After 15 min, mice were anesthetized with isoflurane/oxygen and placed on the imaging stage. Ventral images were collected for 1 min using the Xenogen IVIS imaging system (Xenogen, Alameda, CA, USA). In some experiments, mice were sacrificed, and the organs of interest (kidney, spleen, liver, lung, thymus, intestine) were collected and analyzed using ex vivo imaging techniques. Photons that were emitted from the abdominal region or individual organs were quantified using Living Image software (Xenogen). For experiments with LPS, groups ($n = 10$) containing 12- to 18-week-old male or female Tg mice were injected i.p. with 50 mg/kg of LPS. Control mice were injected with phosphate-buffered saline (PBS). Sixteen hours later, mice were sacrificed, and the organs of interest collected for ex vivo measurements of luciferase activity.

Ex vivo measurement of the luciferase enzymatic activity

Liver, kidney, spleen, lung, and thymus were homogenized in cell culture lysis reagent (CCLR) (Promega) containing phenylmethylsulfoxide (PMSF) at 4°C for 1 h, vortexed for 30 s, snapped frozen in liquid nitrogen and thawed at 37°C. The samples were centrifuged for 20 min at 4°C, and the protein concentration in the supernatant was measured using the Bradford method. Equal amounts of protein were used to analyze the luciferase activity. The luciferase activity was determined using the Luciferase Assay System and a Lumat LB 9507 Luminometer (Berthold Technologies, Oak Ridge, TN, USA).

RNA isolation and semiquantitative PCR

Mouse liver, kidney, spleen, lung, and thymus were excised and immediately frozen at −70°C. Total RNA was isolated

from tissues using Trizol reagent according to the manufacturer's instructions (Invitrogen, Burlington, ON, Canada). Two micrograms of total RNA was reverse transcribed using the Omniscript reverse transcriptase (Qiagen, Mississauga, ON, Canada) and PCR amplified using the following conditions: 94°C for 30 s, 64°C for MMP-9 or 58°C for GAPDH for 1 min, and 72°C for 1 min. A final extension step was performed at 72°C for 10 min. The primers used for PCR amplification were as follows: 5′-CGAGTGGACGCGACCGTAGTTGG-3′ (sense) and 5′-CAGGCTTAGAGCCACGACCATACAG-3′ (antisense) for murine MMP-9 and 5′-CGGAGTCAACGGATTTG GTCGTAT-3′ (sense) and 5′-AGCCTTCTCCATGGTG GTGAAGAC-3′ (antisense) for GAPDH. The amplifications were performed in a thermal cycler (model PTC-100; MJ Research) using equal amounts of RNAs that were reverse transcribed and amplified by PCR for 35 cycles with gene-specific primers. Each amplification was performed in the linear range for each gene. A measure of *GAPDH* mRNA was used as an internal control. The amplified products were analyzed by electrophoresis on 1.2% agarose gels using Sybr Safe staining and UV illumination.

In vitro analyses of Tg leukocytes

Splenocytes were collected from Tg mice and stimulated for 20 h with 100 ng/ml LPS, 1 μg/ml concanavalin A (ConA) (Flow Laboratories, Inglewood, CA, USA) or 15 μg/ml phytohemagglutinin (lectin from *Phaseolus vulgaris*, red kidney bean) (PHA) (Sigma-Aldrich) in 12-well plates. Cells were then collected and analyzed for *mmp-9* promoter activity using the luciferase assay as described above. In some experiments, co-cultured and transwell assays were conducted using splenocytes and B16F1 melanoma cells for 20 h (at ratios of 1:20 and 1:25, respectively) with or without Con A (1 μg/ml) before analysis.

In vivo melanoma model

Eight male Tg mice (8–9 weeks old) were injected subcutaneously (s.c.) with 10^4 B16F1 melanoma cells in the left flank. Animals were carefully monitored periodically for tumor growth. The length (L) and width (W) of the tumor were measured using a Vernier caliper. The size of the tumor was calculated using the formula $L^2 \times W \times 0.4$. When the maximum tumor volume was reached, which occurred after 19–22 days post-injection, all of the mice were injected i.p. with 150 mg/kg of D-luciferin and were sacrificed after 10 min to collect the lungs and spleen for ex vivo imaging. The homogenates were then prepared for the luciferase assay and the RT-PCR analysis as described above. The dissemination of melanoma cells in target organs was analyzed using s.c injection of stable transfectants of B16F1-melanoma cells (5×10^4 cells) that constitutively expressed the *luciferase* reporter gene under the control of the SV40 promoter in C57BL/6 female mice (6 weeks old). Normal C57BL/6 mice were used as controls.

Statistical analysis

Data are represented as means ± SD. Student's t test was used to test for statistical significance, and the level of significance was established at $P < 0.05$.

Results

Generation of transgenic mice containing the *mmp*-9 promoter-driven luciferase reporter transgene

The plasmid used for the construction of the C57BL/6-Tg(*proMMP9-Luc*) mice has been previously described [8] (Fig. 1a). This 681-bp fragment contains the consensus binding motifs that are common to both the murine and human genes and that are essential for the transcriptional activity of the murine *mmp-9* promoter, including a NF-κB binding site located at position −600 bp. Tg mouse founders were identified by PCR detection of the Tg *luciferase* gene in tail-tip DNA and backcrossed to C57BL/6 mice for at least five generations to generate progeny. The insertion of the transgene was confirmed by PCR genotyping using primers that were specific for the *mmp-9* promoter and the *luciferase* reporter gene (Fig. 1b). To determine the expression pattern of the transgene in vivo, bioluminescent imaging was performed in living Tg animals and compared to non-Tg mice. Our results show that all C57BL/6-Tg(*proMMP9-Luc*) mice displayed light emissions in the abdomen and the lower extremities of the limbs (Fig. 1c). No background signal was detected in the non-Tg control mice. Ex vivo imaging demonstrated that the high basal expression of the transgene in the abdominal cavity most likely originated from the intestine. The basal luminescence was also consistently observed in the thymus, whereas the occasional expression of the transgene was detected in the spleen and lung (Fig. 2a). No detectable basal expression of the transgene was found in the kidneys and the liver (Fig. 2a). These results were consistent with those obtained using luciferase assays of tissue extracts (Fig. 1d) and analyses from the *genomic profile arrays* of C57BL/6 tissues [9]. A constitutive transgene expression was also observed in the bone marrow from Tg mice (Fig. 1e), which is consistent with the high levels of *mmp-9* gene expression normally found in the bone marrow (Fig. 1f) [7, 10].

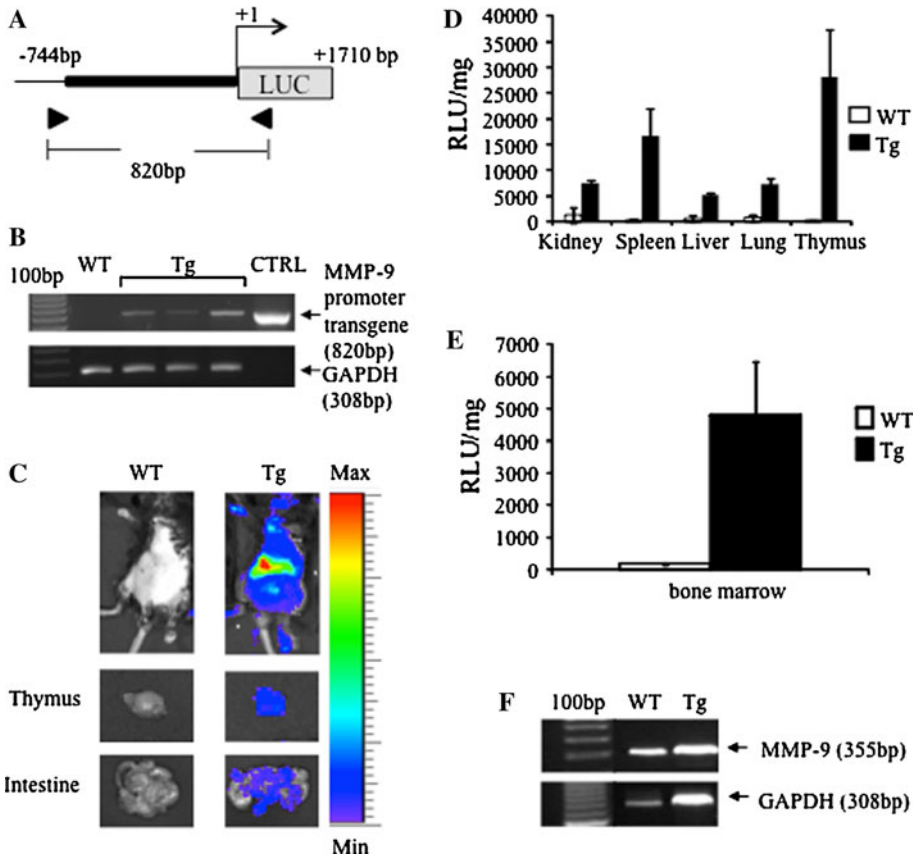

Fig. 1 Generation of Tg mice containing the *mmp-9* promoter-driven luciferase reporter transgene. **a** Schematic illustration of the *mmp-9* promoter construct with the *luciferase* reporter gene; the *symbols* (*right pointing filled triangle*, *left pointing filled triangle*) represent the primers used for genotyping. **b** PCR genotype analysis was performed using the primers that are shown in (**a**) for the *mmp-9* promoter transgene detection in C57BL/6-Tg(*proMMP9-Luc*) mice in comparison to that in control C57BL/6 mice (*WT*) and a positive control mouse containing the PGL3-MMP-9 vector (*CTRL*). **c** The visualization of the luciferase expression of a representative C57BL/6-Tg(*proMMP9-Luc*) mouse and its organs (thymus and intestine) in comparison to those of a control mouse using the Xenogen IVIS imaging system. The luciferase assay, which was represented as relative light units (RLU) per mg of protein, was performed on **d** the kidney, the spleen, the liver, the lung, the thymus and **e** the bone marrow of C57BL/6-Tg(*proMMP9-Luc*) mice (*filled square*) ($n = 9$) in comparison to those of control mice (*open square*) ($n = 9$). **f** RT-PCR analysis demonstrating the increased endogenous MMP-9 mRNA expression in the bone marrow of a representative C57BL/6-Tg(*proMMP9-Luc*) mouse in comparison to that in the bone marrow of a control mouse. GAPDH was used as a loading and specificity control

Functional analysis of the *mmp*-9 promoter transgene

MMP-9 gene expression is regulated by a number of different stimuli in different cell types, most notably by LPS in liver and kidney tissues [11–13]. To determine whether our transgene model may be used to assess the activation of the *mmp-9* promoter by inflammatory mediators using bioluminescence imaging, we injected Tg and control mice with LPS. Sixteen hours after the LPS injection, the activation of the *mmp-9* promoter was examined using ex vivo imaging of the target organs. We found that LPS injection (50 mg/kg) induced a consistent and significant increase in bioluminescent signals in the liver (Fig. 2a), which is a major target of LPS-induced septic shock [13]. No significant or consistent increase in bioluminescence was found in the other organs, such as the spleen, the kidneys, the lung, and the thymuses, following LPS injection. However, ex vivo analyses using the luciferase assay revealed the activation of the *mmp-9* Tg promoter in the liver and the kidneys ($P = 0.0001$ and $P = 0.0015$, respectively) (Fig. 2b). These results indicate that the ex vivo luciferase assay is more sensitive than the ex vivo measures of bioluminescence. The activation of the *mmp-9* Tg promoter by LPS in the liver and kidneys was consistent with the activation of the endogenous *mmp-9* gene (Fig. 2c). Although no significant increase in the overall signal was demonstrated using ex vivo imaging of the spleen following LPS injection, we identified a significant ($P = 0.04$) activation of the *mmp-9* transgene in isolated splenocytes that were treated with T cell-specific mitogens such as Con A and PHA, indicating that the transgene is also functional in T lymphocyte populations (Fig. 2d).

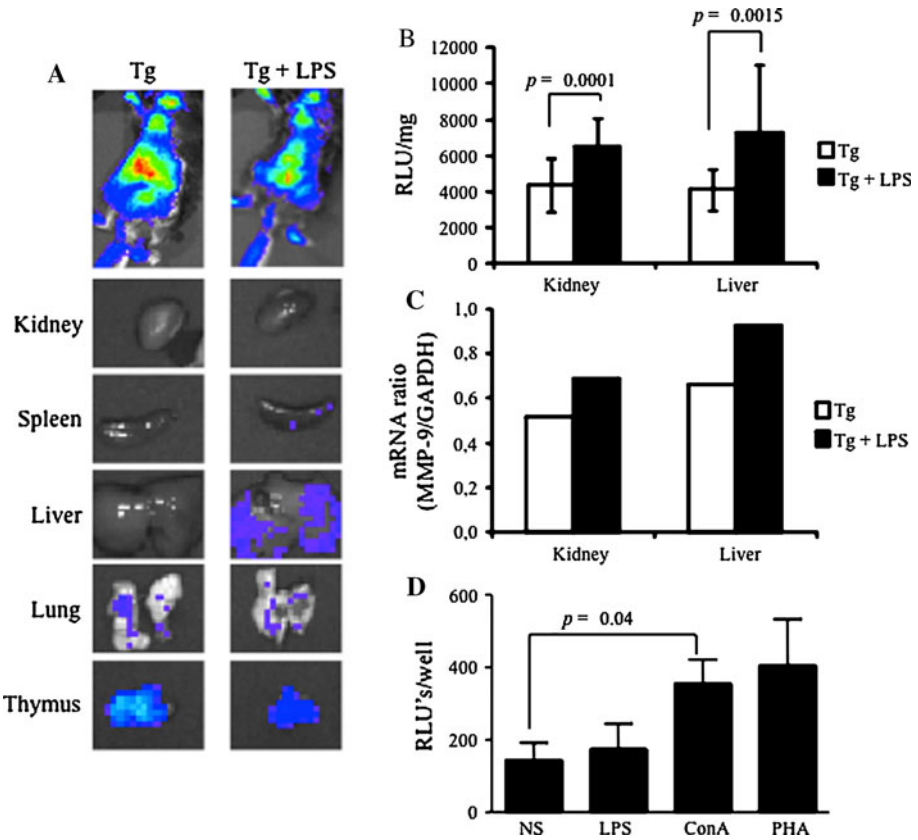

Fig. 2 In vivo activation of the *proMMP9-Luc* transgene by LPS. **a** Luciferase activity in C57BL/6Tg(*proMMP9-Luc*) mice and organs (kidney, spleen, liver, lung and thymus) 16 h after i.p. injection of two mg/kg of LPS. The controls included C57BL/6-Tg(*proMMP9-Luc*) mice injected with PBS. **b** Ex vivo analysis of the luciferase activity in tissues that were collected from Tg mice that were injected with 50 mg/kg of LPS (*filled square*; $n = 10$) or PBS (*open square*; $n = 9$). **c** Endogenous levels of MMP-9 measured by RT-PCR 16 h post-injection of two mg/kg of LPS (*filled square*) or PBS (*open square*). GAPDH was used as a loading and specificity control. Relative *mmp-9* levels are expressed as a ratio of *mmp-9/GAPDH*. **d** Luciferase assay on splenocytes collected from C57BL/6-Tg(*proMMP9-Luc*) mice 20 h after stimulation with LPS (100 ng/ml). Non-stimulated splenocytes were used as controls (*NS*). Results represent the mean of two independent experiments

Directional interactions between tumor cells and stromal cells induce the secretion of MMP-9 in the extracellular space of the tumor microenvironment [4, 6, 14]. Accordingly, it is difficult to distinctively determine the *mmp-9* gene activation in a specific cell population. Therefore, we examined whether our C57BL/6-Tg(*proMMP9-Luc*) mice could be used to overcome this limitation. For this purpose, C57BL/6-Tg(*proMMP9-Luc*) mice were injected s.c. with B16F1 melanoma cells, and the activation of the *mmp-9* transgene in the organs of tumor-bearing mice was measured by in vivo imaging and the ex vivo luciferase assay. We did not detect a significant increase in the luciferase signal using whole-body imaging. However, the activation of the transgene was observed using ex vivo imaging of the lungs of tumor-bearing C57BL/6-Tg(*proMMP9-Luc*) mice at day 19 post-injection of B16F1 melanoma cells (Fig. 3a). This increase was confirmed using ex vivo measurements of the luciferase activity of lung extracts (Fig. 3b), which was consistent with an increase of the endogenous MMP-9 mRNA levels in the lungs of C57BL/6Tg(*proMMP9-Luc*) mice that were injected with B16F1 melanoma cells (Fig. 3c). A significant increase in the luciferase activity was also observed in the spleens of tumor-bearing mice compared to that in the spleens of control C57BL/6-Tg(*proMMP9-Luc*) mice (Fig. 4a). This increase was consistent with the ability of the B16F1 cells to migrate into the spleen of tumor-bearing animals (Fig. 4b). No such increase was observed in the kidneys or the liver. A luciferase assay was conducted using splenocytes collected from C57BL/6-Tg(*proMMP9-Luc*) and B16F1 melanoma cells that were co-cultured for 20 h with or without Con A, which is a T cell-specific mitogen known to potentiate cellular activation. The results suggest that at least part of the increase in luciferase activity may originate from the T cell activation in the spleen of tumor-bearing animals (Fig. 5a) [15]. Experiments using transwells showed that the contact between the B16F1 melanoma cells and the spleen cells was not essential to

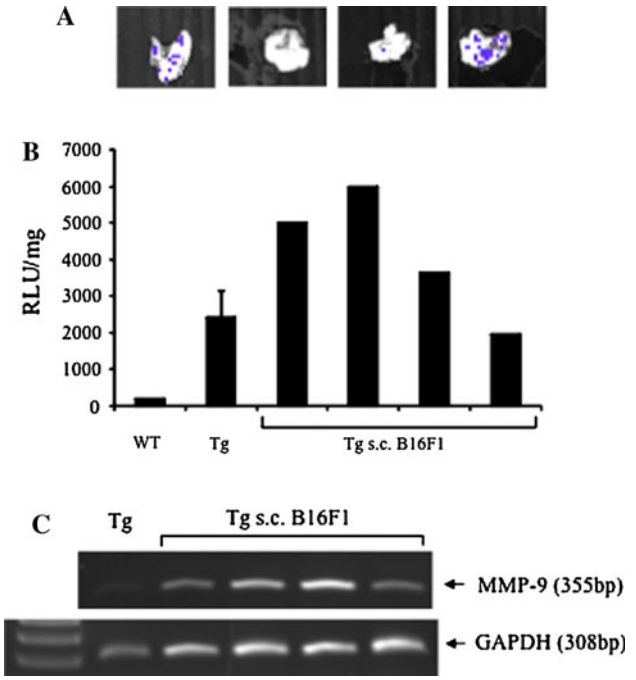

Fig. 3 Activation of the *proMMP9-Luc* transgene in the lung. C57BL/6Tg(*proMMP9-Luc*) mice were injected s.c. with 10^4 B16F1 melanoma cells. Nineteen days post-injection, lungs were collected, and the individual mice were analyzed ex vivo using **a** bioluminescent imaging or **b** the luciferase activity assay. **c** Increased expression of the endogenous MMP-9 in lungs collected from C57BL/6-Tg(*proMMP9-Luc*) mice injected with B16F1 melanoma cells. Lungs collected from non-injected C57BL/6-Tg(*proMMP9-Luc*) mice were used as controls. GAPDH was used as a loading and specificity control

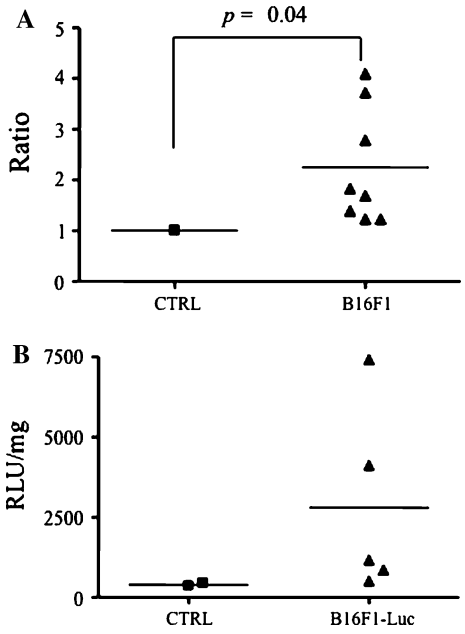

Fig. 4 Activation of the *proMMP9-Luc* transgene in the spleen of tumor-bearing animals. C57BL/6-Tg(*proMMP9-Luc*) mice were injected with 10^4 B16F1 melanoma cells. At 20–22 days post-injection, spleens were collected and luciferase activity measured from tissue extracts. **a** Luciferase activity in spleens from tumor-bearing Tg animals injected with *B16F1* cells (*filled triangle*; $n = 8$) compared to spleens from control animals (*CTRL*; *filled square*; $n = 4$). Results are representative of two independent experiments. **b** Luciferase activity from spleen extracts prepared from normal C57BL/6 mice injected with B16F1-Luc melanoma cells (*B16F1-Luc*; *filled triangle*). Spleens from control age- and sex-matched C57BL/6 animals were used as controls (*CTRL*; *filled square*)

significantly increase the *mmp-9* transgene expression in splenocytes (Fig. 5b). These results indicate that the soluble factors that are produced by the B16F1 melanoma cells may potentiate the Con A-induced T cell activation. Taken together, these results indicate that the *proMMP9-Luc* transgene is functional and that C57BL/6-Tg(*proMMP9-Luc*) mice can be used to examine the activation of stromal cells in response to tumor growth.

MMP-9 is expressed by osteoclasts in the bone, and it plays an important role in bone formation and arthritis [16–19]. The whole-body imaging of our C57BL/6-Tg(*proMMP9-Luc*) mice revealed a consistent and constitutive activation of the *proMMP9-Luc* transgene in paws (Figs. 1c and 2a). To examine the usefulness of our Tg model in arthritis, male and female C57BL/6-Tg(*proMMP9-Luc*) mice were treated with dexamethasone (DXM; i.p.; 0.5 mg/kg/day) for 3 weeks (Fig. 6a). The control mice were injected with PBS for 3 weeks. The bioluminescent imaging of individual paws was performed before the injection ($T = 0$) and at days 5 and 19 post-treatment. Our results demonstrate that 60–75% of the paws from the DXM-treated mice show a downregulation of the transgene expression after 5 and 19 days of treatment with DXM compared to 30% of the paws from control mice (Fig. 6b, c). No decrease in the transgene expression was observed in the spleen and the bone marrow of Tg mice that were untreated or treated with DXM, which were analyzed using the luciferase assay after 19 days (data not shown). These results suggest that C57BL/6-Tg(*proMMP9-Luc*) mice may be useful to assess the potential of immunosuppressive drugs.

Discussion

In the present work, we have generated a Tg mouse model expressing the *luciferase* reporter gene under the control of the murine *mmp-9* promoter. The expression pattern of the transgene was similar to the expression pattern of the endogenous *mmp-9* gene under constitutive activation or following stimulation with inflammatory mediators. We demonstrated that the transgene was activated in the lung and the spleen of melanoma tumor-bearing mice. These results suggest that this Tg mouse model may be useful to

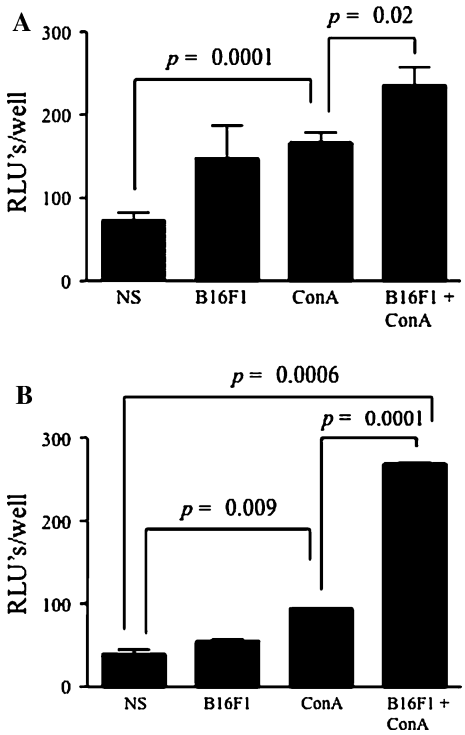

Fig. 5 In vitro stimulation of the *proMMP9-Luc* transgene in splenocytes. **a** Splenocytes were collected from C57BL/6-Tg(*proMMP9-Luc*) mice and co-cultured ($n = 6$) with *B16F1* melanoma cells for 20 h with or without *Con A* (1 μg/ml). **b** Transwell experiments using co-culture conditions as described in (**a**). Results are representative of three independent experiments

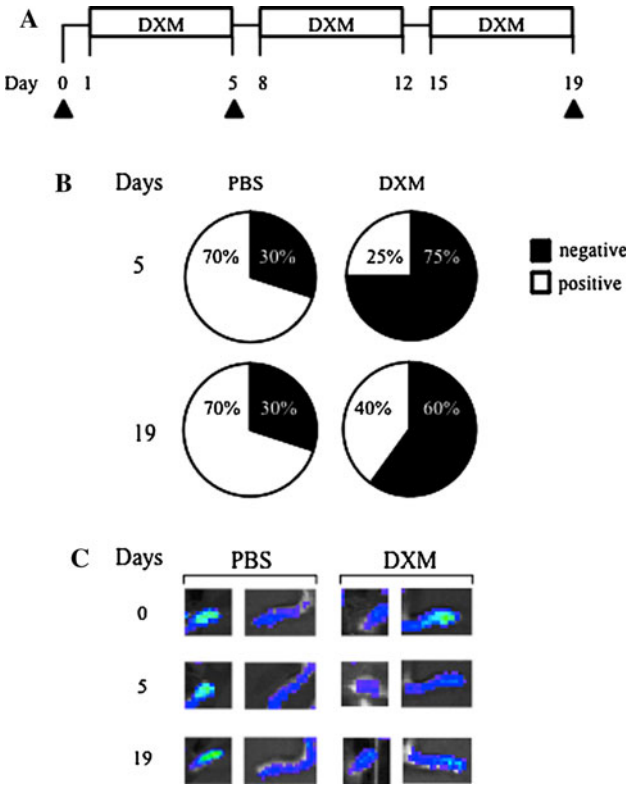

Fig. 6 Effect of dexamethasone (DXM) on MMP-9 expression in paws. **a** Schematic representation of the treatment regimen using i.p. injections of *DXM* (0.5 mg/kg/day). Bioluminescent imaging of the luciferase activity was performed at days 5 and 19. **b** *Pie chart* representation of the response of C57BL/6-Tg(*proMMP9-Luc*) mice to *DXM* as compared to that of the control mice injected with *PBS*. Results are representative of triplicate images acquired for each paw before ($T = 0$) and at different times after the initial treatment with DXM ($n = 20$) or control PBS ($n = 20$). **c** In vivo imaging of the *proMMP9-Luc* transgene expression in two representative paws of C57BL/6Tg*(proMMP9-Luc)* mice injected with *PBS* (Control) or *DXM*

identify the cell population(s) that expresses MMP-9 in the context of bi-directional interactions between cancer cells and peritumoral stromal cells. This is an important feature because determining whether cancer cells and/or stromal cells secrete MMP-9 at different steps of cancer progression has been a major challenge [4, 6, 14].

LPS is known for its ability to activate host-inflammatory responses by inducing the release of pro-inflammatory cytokines such as TNF-α and interleukin-1β [14, 20]. These cytokines induce *mmp-9* gene expression via NF-κB, which is a critical factor that regulates *mmp-9* gene expression [21, 22]. Our results show that LPS increased the luciferase activity in liver and kidney tissues and are, therefore, consistent with previous studies using NF-κB-*Luc* Tg models, which demonstrated that the NF-kB binding site is activated by LPS in these organs [23]. This model may be useful to study the expression of MMP-9 in inflammatory processes. In addition, our results illustrate that DXM, which is a powerful anti-inflammatory agent, induces a significant suppression of the transgene in the paws of Tg mice. This bioluminescent imaging model may facilitate the repeated measures of *mmp-9* gene activation in living mice and may be used to test novel anti-inflammatory drugs.

The expression of MMP-9 by tumor cells plays a critical role in tumor progression [1]. In addition, a significant number of studies have shown that the secretion of MMP-9 by peritumoral cells may equally contribute to tumor growth and metastasis, especially in melanoma [4, 24–28]. However, it has been relatively difficult to study the *mmp-9* gene expression in stromal cells in response to tumor cells in vivo because *mmp-9* is often expressed by the tumor cells themselves. Therefore, the ability of tumor cells to induce MMP-9 in peritumoral cells has been mostly established using in vitro model systems [14, 29–31]. In the case of melanoma, the increases in *mmp-9* gene expression in melanoma cells are well described [32, 33]. However, the ability of melanoma cells to induce stromal *mmp-9* gene expression in vivo remains unclear. Our results in tumor-bearing Tg mice provide new evidence that tumor cells induce *mmp-9* gene expression in the peritumoral environment.

Animal models are important tools to better understand the molecular mechanisms underlying pathogenesis and to develop novel therapeutic approaches for human diseases. Given the importance of MMP-9 in disease progression, Mohan et al. [34] designed a minimal *mmp-9* promoter to generate a similar Tg mouse model using LacZ to facilitate the localization of *mmp-9*-expressing cells. This model was useful to study the developmental regulation of *mmp-9* in tissue sections in the complex environment of the embryo using β-gal staining. This model used a shorter construct containing a 522-bp stretch of the 5′ flanking region of the rabbit *mmp-9* gene. Here, we used a well-characterized promoter sequence containing a 681-bp stretch of the 5′ flanking region of the mouse *mmp-9* gene. Our Tg mouse model is thus different at the molecular level from that of Mohan et al. Our model is also complementary because luciferase reporter systems are ideal for continuous real-time measurement of bioluminescence signals emitted from firefly luciferase-based reporter system under the control of specific mammalian promoters using a non-invasive imaging modality and standard bioimaging equipment [35–37]. Our *proMMP9-Luc* Tg mouse model does have some limitations. For example, the high bioluminescence originating from the abdominal space complicates in vivo studies on specific abdominal organs. In these cases, transgene expression requires ex vivo imaging. Measuring the ex vivo luciferase activity in tissue extracts is also a valid alternative, which we found to be more sensitive and quantitative than the ex vivo imaging. Therefore, both models may be used in conjunction with transcriptional inhibitors to identify novel modulators of *mmp-9* gene expression in inflammatory disorders and cancer.

Our results have shown a significant luciferase activity in the bone marrow. MMP-9 expression by bone marrow-derived cells plays an important role in the survival of tumor cells in the lung microenvironment [38]. This is particularly true for neutrophils which are the predominant source of MMP-9 for the establishment of pheripheral metastasis of breast cancer cells [39]. Interestingly, a suppression of MMP9 in the bone marrow correlates with a concomitant decrease in tumor number, indicating that our transgenic mouse model would be useful to test the effects of new drugs on MMP-9 gene expression in the the bone marrow [39]. Such a beneficial effect of MMP-9 suppression has also been observed in the targeting of splenocytes in EAE models, indicating that inhibition of MMP-9 is a promising treatment in patients with MS.

In summary, we have developed a new Tg mouse model to study the *mmp-9* gene expression in normal and pathological conditions using luciferase-based bioluminescent imaging. This model may be an ideal foundation for the establishment of biogenic imaging of mouse models to study *mmp-9* gene expression during disease progression.

Acknowledgments We thank Diane Tremblay for her excellent technical support. K.B.P. was supported by a doctoral studentship from the Fonds de la Recherche en Santé du Québec (FRSQ). This work was supported by the Cancer Research Society of Canada.

References

1. Lopez-Otin C, Matrisian LM (2007) Emerging roles of proteases in tumour suppression. Nat Rev Cancer 7:800–808. doi:10.1038/nrc2228
2. Coussens LM, Fingleton B, Matrisian LM (2002) Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 295:2387–2392. doi:10.1126/science.1067100
3. Overall CM, Kleifeld O (2006) Towards third generation matrix metalloproteinase inhibitors for cancer therapy. Br J Cancer 94:941–946. doi:10.1038/sj.bjc.6603043
4. Coussens LM, Tinkle CL, Hanahan D, Werb Z (2000) MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell 103:481–490. doi:10.1016/S0092-8674(00)00139-2
5. Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D (2000) Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2:737–744. doi:10.1038/35036374
6. Itoh T, Tanioka M, Matsuda H, Nishimoto H, Yoshioka T, Suzuki R, Uehira M (1999) Experimental metastasis is suppressed in MMP-9-deficient mice. Clin Exp Metastasis 17:177–181. doi:10.1023/A:1006603723759
7. Acuff HB, Carter KJ, Fingleton B, Gorden DL, Matrisian LM (2006) Matrix metalloproteinase-9 from bone marrow-derived cells contributes to survival but not growth of tumor cells in the lung microenvironment. Cancer Res 66:259–266. doi:10.1158/0008-5472.CAN-05-2502
8. Esteve PO, Chicoine E, Robledo O, Aoudjit F, Descoteaux A, Potworowski EF, St-Pierre Y (2002) Protein kinase C-zeta regulates transcription of the matrix metalloproteinase-9 gene induced by IL-1 and TNF-alpha in glioma cells via NF-kappa B. J Biol Chem 277:35150–35155. doi:10.1074/jbc.M108600200
9. Thorrez L, Van Deun K, Tranchevent LC, Van Lommel L, Engelen K, Marchal K, Moreau Y, Van Mechelen I, Schuit F (2008) Using ribosomal protein genes as reference: a tale of caution. PLoS ONE 3:e1854. doi:10.1371/journal.pone.0001854
10. Aoudjit F, Esteve PO, Desrosiers M, Potworowski EF, St-Pierre Y (1997) Gelatinase B (MMP-9) production and expression by stromal cells in the normal and adult thymus and experimental thymic lymphoma. Int J Cancer 71:71–78. doi:10.1002/(SICI)1097-0215(19970328)
11. Opdenakker G, Masure S, Grillet B, Van Damme J (1991) Cytokine-mediated regulation of human leukocyte gelatinases and role in arthritis. Lymphokine Cytokine Res 10:317–324
12. Renckens R, Roelofs JJ, Florquin S, de Vos AF, Lijnen HR, van't Veer C, van der Poll T (2006) Matrix metalloproteinase-9 deficiency impairs host defense against abdominal sepsis. J Immunol 176:3735–3741
13. Murch O, Collin M, Sepodes B, Foster SJ, Mota-Filipe H, Thiemermann C (2006) Lysophosphatidylcholine reduces the organ injury and dysfunction in rodent models of gram-negative and gram-positive shock. Br J Pharmacol 148:769–777. doi:10.1038/sj.bjp.0706788
14. Aoudjit F, Potworowski EF, St-Pierre Y (1998) Bi-directional induction of matrix metalloproteinase-9 and tissue inhibitor of

matrix metalloproteinase-1 during T lymphoma/endothelial cell contact: implication of ICAM-1. J Immunol 160:2967–2973
15. Kuvibidila SR, Gardner R, Velez M, Yu L (2010) Iron deficiency, but not underfeeding reduces the secretion of interferon-gamma by mitogen-activated murine spleen cells. Cytokine 52:230–237. doi:10.1016/j.cyto.2010.08.004
16. Ortega N, Wang K, Ferrara N, Werb Z, Vu TH (2010) Complementary interplay between matrix metalloproteinase-9, vascular endothelial growth factor and osteoclast function drives endochondral bone formation. Dis Model Mech 3:224–235. doi:10.1242/dmm.004226
17. Reponen P, Sahlberg C, Munaut C, Thesleff I, Tryggvason K (1994) High expression of 92-kD type IV collagenase (gelatinase B) in the osteoclast lineage during mouse development. J Cell Biol 124:1091–1102
18. Yoshida W, Uzuki M, Nishida J, Shimamura T, Sawai T (2009) Examination of in vivo gelatinolytic activity in rheumatoid arthritis synovial tissue using newly developed in situ zymography and image analyzer. Clin Exp Rheumatol 27:587–593
19. Gruber BL, Sorbi D, French DL, Marchese MJ, Nuovo GJ, Kew RR, Arbeit LA (1996) Markedly elevated serum MMP-9 (gelatinase B) levels in rheumatoid arthritis: a potentially useful laboratory marker. Clin Immunol Immunopathol 78:161–171. doi:10.1006/clin.1996.0025
20. Zhou H, Bernhard EJ, Fox FE, Billings PC (1993) Induction of metalloproteinase activity in human T-lymphocytes. Biochim Biophys Acta 1177:174–178. doi:10.1016/0167-4889(93)90037-P
21. Xie B, Dong Z, Fidler IJ (1994) Regulatory mechanisms for the expression of type IV collagenases/gelatinases in murine macrophages. J Immunol 152:3637–3644
22. Arechavaleta-Velasco F, Ogando D, Parry S, Vadillo-Ortega F (2002) Production of matrix metalloproteinase-9 in lipopolysaccharide-stimulated human amnion occurs through an autocrine and paracrine proinflammatory cytokine-dependent system. Biol Reprod 67:1952–1958. doi:10.1095/biolreprod.102.004721
23. Carlsen H, Moskaug JO, Fromm SH, Blomhoff R (2002) In vivo imaging of NF-kappa B activity. J Immunol 168:1441–1446
24. Ruiter D, Bogenrieder T, Elder D, Herlyn M (2002) Melanoma-stroma interactions: structural and functional aspects. Lancet Oncol 3:35–43
25. Giraudo E, Inoue M, Hanahan D (2004) An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. J Clin Investig 114:623–633. doi:10.1172/JCI22087
26. Ahn GO, Brown JM (2008) Matrix metalloproteinase-9 is required for tumor vasculogenesis but not for angiogenesis: role of bone marrow-derived myelomonocytic cells. Cancer Cell 13:193–205. doi:10.1016/j.ccr.2007.11.032
27. Marconi C, Bianchini F, Mannini A, Mugnai G, Ruggieri S, Calorini L (2008) Tumoral and macrophage uPAR and MMP-9 contribute to the invasiveness of B16 murine melanoma cells. Clin Exp Metastasis 25:225–231. doi:10.1007/s10585-007-9136-0
28. Lin EY, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, Qian H, Xue XN, Pollard JW (2006) Macrophages regulate the angiogenic switch in a mouse model of breast cancer. Cancer Res 66:11238–11246. doi:10.1158/0008-5472.CAN-06-1278
29. Shekhar MP, Werdell J, Santner SJ, Pauley RJ, Tait L (2001) Breast stroma plays a dominant regulatory role in breast epithelial growth and differentiation: implications for tumor development and progression. Cancer Res 61:1320–1326
30. Stuelten CH, DaCosta Byfield S, Arany PR, Karpova TS, Stetler-Stevenson WG, Roberts AB (2005) Breast cancer cells induce stromal fibroblasts to express MMP-9 via secretion of TNF-alpha and TGF-beta. J Cell Sci 118:2143–2153. doi:10.1242/jcs.02334
31. Voigt H, Houben R, Schrama D, Hofmann UB, Vetter-Kauczok CS, Becker JC (2007) Matrix metalloproteinase induction in the tumor stroma does not depend on CD147 expression in murine B16 melanoma. Tumour Biol 28:229–237. doi:000107418[pii]10.1159/000107418
32. MacDougall JR, Bani MR, Lin Y, Rak J, Kerbel RS (1995) The 92-kDa gelatinase B is expressed by advanced stage melanoma cells: suppression by somatic cell hybridization with early stage melanoma cells. Cancer Res 55:4174–4181
33. Hofmann UB, Houben R, Brocker EB, Becker JC (2005) Role of matrix metalloproteinases in melanoma cell invasion. Biochimie 87:307–314. doi:10.1016/j.biochi.2005.01.013
34. Mohan R, Rinehart WB, Bargagna-Mohan P, Fini ME (1998) Gelatinase B/lacZ transgenic mice, a model for mapping gelatinase B expression during developmental and injury-related tissue remodeling. J Biol Chem 273:25903–25914. doi:10.1074/jbc.273.40.25903
35. Gross S, Piwnica-Worms D (2005) Real-time imaging of ligand-induced IKK activation in intact cells and in living mice. Nat Methods 2:607–614. doi:10.1038/nmeth779
36. Gross S, Piwnica-Worms D (2005) Spying on cancer: molecular imaging in vivo with genetically encoded reporters. Cancer Cell 7:5–15. doi:10.1016/j.ccr.2004.12.011
37. Grossman E, Tao J, Wilson R, Park SY, Bell G, Chong A (2010) Seeing is believing: how the MIP-luc mouse can advance the field of islet transplantation and β-cell regeneration. Islets 2:261–262. doi:10.4161/isl.2.4.12028
38. Welch DR, Schissel DJ, Howery RP, Aeed PA (1989) Tumor-elicited polymorphonuclear cells, in contrast to "normal" circulating polymorphonuclear cells, stimulate invasive and metastatic potentials of rat mammary adenocarcinoma cells. Proc Natl Acad Sci USA 86:5859–5863
39. Acuff HB, Carter KJ, Fingleton B, Gorden DL, Matrisian LM (2006) Matrix metalloproteinase-9 from bone marrow-derived cells contributes to survival but not growth of tumor cells in the lung microenvironment. Cancer Res 66:259–266. doi:10.1158/0008-5472.CAN-0502502