ONCOLOGY

Changes in Tissue Metalloproteinase Inhibitor-1 and Matrix Metalloproteinases during Tumor Development and Metastasizing in Mice

T. A. Korolenko, T. G. Filatova, V. M. Belichenko, T. V. Alexeenko, and S. Ya. Zhanaeva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 8, pp. 198-201, August, 2010
Original article submitted April 21, 2009

Tissue inhibitor of matrix metalloproteinases type 1, inhibiting the majority of matrix metalloproteinases, can both suppress and stimulate tumor growth. The concentrations and activities of tissue matrix metalloproteinase inhibitor-1 were measured in C57Bl/6 mice during progression and metastasizing of Lewis lung adenocarcinoma. Activities of matrix metalloproteinases in tumor tissue of mice were lower than in liver and lung tissues of intact animals. Serum concentration of tissue inhibitor increased significantly during the development of Lewis lung adenocarcinoma. Macrophage depression (injection of gadolinium chloride associated with a decrease in metastasis number) decreased serum concentration of tissue inhibitor, but it did not attain the control level observed in intact mice. These findings attest to a pleiotropic antitumor effect of tissue matrix metalloproteinase inhibitor-1 reflecting disorders in matrix metalloproteinase regulation during the progress of Lewis lung adenocarcinoma in mice.

Key Words: *tissue inhibitor-1 of matrix metalloproteinases; matrix metalloproteinases; mouse tumors; metastasizing*

Intensification of degradation and remodeling of extracellular matrix components plays an important role in tumor progress [1,2] starting from the stage of basal membrane destruction by primary tumor to the development of metastases [3,4]. Matrix metalloproteinases (MMP) are expressed and secreted by various tumor cells and macrophages associated with the tumor and involved in the extracellular matrix remodeling [7-9]. Tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) is one of four MMP inhibitors (matrixines), enzymes destroying the majority of extracellular matrix components. Secreted TIMP form complexes with active MMP or their proforms, reducing MMP activities and modifying the MMP/TIMP balance (for example, MMP-2/TIMP-1). In recent studies, TIMP-1 is regarded as a multifunctional protein playing an ambiguous complex role in tumor development [10,12-14]. Antiapoptotic effect of TIMP-1 was detected in some tumors. It is attributed to resistance to antitumor therapy [5,15]. This is confirmed by high sensitivity of fibrosarcoma cells of TIMP-1 gene-deficient mice to antitumor therapy [5]. However, the role of TIMP-1 changes in the regulation of activities of various MMP types during tumor development and metastasizing and the relationship between these changes and macrophage stimulation remain unclear.

Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** T.A.Korolenko@physiol.ru. T. A. Korolenko

0007-4888/10/1502-0233

We evaluated the role of changes in TIMP-1 and MMP activity in the development and metastasizing of tumors under conditions of macrophage depression *in vivo*.

MATERIALS AND METHODS

The study was carried out on male C57Bl/6 mice (25-30 g; vivarium of Institute of Cytology and Genetics), to which Lewis lung carcinoma (LLC), metastasizing into the lungs [1] was transplanted. Liver macrophage depression was induced by intraperitoneal injection of gadolinium chloride ($GdCl_3$) in a single dose of 14 mg/kg. A higher dose of $GdCl_3$ (28 mg/kg) was used for suppressing liver and lung macrophage pools [1,2]. Gadolinium chloride was injected to animals on days 3 or 8 (beginning of metastasizing) after tumor transplantation. The animals were decapitated 11, 13, 15, 17, and 20 days after tumor transplantation. Preparative procedures and tumor, liver, and lung tissue homogenate preparation were described previously [1,2]. The serum was separated by centrifugation of the samples at 3000*g* and 4°C for 20 min (Eppendorf 5415 R centrifuge).

Serum concentration of TIMP-1 was measured by EIA using commercial kits for mice (RayBiotech Inc.). The sensitivity of the method is <3 pg/ml, it is highly specific (no cross reactions with cytokines and adhesion proteins). Mouse recombinant TIMP-1 served as the reference sample. The results were expressed in pg/ml serum. Serum concentration of MMP-2 was measured by solid phase EIA with commercial kits for MMP-2 measurements in human/rat/mouse biological fluids (R&D). This EIA version can be used for measurements of the concentrations of active MMP-2 and its proform without cross reactions with other MMP types. The reference sample was MMP-2. Extinction of the samples was evaluated using MULTISCAN EX plate reader (Thermo Electron Corp.) at λ=450 nm. The results were expressed in ng/ml serum. The samples were standardized by cellular protein content before measurements of TIMP-1 and MMP-2 concentrations.

Total MMP activity was measured against the MCA-Pro-Leu-Gly-Leu-DpA-Ala-Arg-NH_2 substrate (American Peptide Co.) at pH 7.5 [7,12]. Using this peptide substrate with fluorescence quencher, mainly MMP-2 (gelatinase A) and MMP-7 (matrilysin) activities are detected in the molecule structure, while a modern proteomic analysis evaluates the total activities of the majority of MMP types [7,8]. Fluorescence was evaluated using Shimadzu RF-5301PC spectrofluorometer at λ=325 (stimulation) and λ=390 (emission). Methylcoumarylamide (MCA; Sigma) served as the reference sample. The results were expressed in μmol MCA/min/mg protein.

The data were processed by variation statistics parallel series method using SPSS 9.0 software and Student's *t* test. The differences between the means were considered significant at $p<0.05$.

RESULTS

Changes in MMP activity during LLC growth and metastasizing in mice. MMP activity in tumor tissue (days 20 after transplantation) was lower than in intact mouse lungs (organ from which LLC originated) and liver (reference organ, Fig. 1). Low MMP activity was also found in tumor tissue in comparison with intact mouse lungs on days 13 and 17 after transplantation. Injection of GdCl3 did not change tumor MMP activity (Fig. 2).

Activities of MMP in lung tissue with metastases decreased on days 13, 17, and 20 in mice injected and not injected with $GdCl_3$ in comparison with the lungs of intact animals (Fig. 2). Injection of $GdCl_3$ to intact animals did not change MMP activity in the lungs (Fig. 2). Low activity of MMP presumably reflects the predominance of inert MMP proforms (presumably pro-MMP-2 and pro-MMP-9) in tumor tissue and in lung tissue with tumor metastases. Inert MMP proforms without enzymatic activity were detected in many tumors by zymography allowing differentiated evaluation of MMP-2 and MMP-9 gelatinase proforms and active forms [3,4,7].

Contrary to this, a moderate significant elevation of total MMP-2 (active form and proenzyme) concentration in the serum was seen on day 13 of tumor development ($p<0.05$ *vs.* intact animals; Table 1). Presumably, this elevation was due to more intense secretion of the mature and immature MMP-2 forms noted

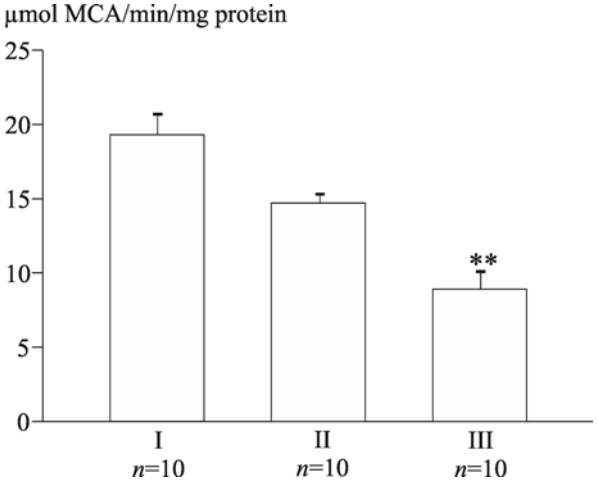

Fig. 1. Total MMP activity in the lungs and liver of intact mice and in tumor tissue from mice with LLC. I: lungs (intact); II: liver (intact), III: tumor, day 20 after transplantation. ***p*<0.01 compared to MMP activity in the lungs and liver of intact animals.

in some tumors [8]. Later (day 17) serum MMP-2 concentration did not differ from that in intact mice (Fig. 2). Injection of $GdCl_3$ in a dose of 14 mg/kg reduced MMP-2 concentration in intact animals and in animals with LLC on day 13 (in comparison with mice with LLC which received no gadolinium during the same period of tumor development); 17 days after tumor transplantation the changes were significant only in comparison with intact mice (Table 1). It seems that $GdCl_3$ inhibited tumor development as we had shown previously [1,2], partially at the expense of inhibition of MMP-2 involved in angiogenesis and lysis of cell surface proteins [8].

Changes in TIMP-1 concentration during LLC development in mice. The development of tumor during all periods (days 11, 13, 15, 17, and 20 after transplantation) was paralleled by a significant increase of TIMP-1 concentration in the serum (Fig. 3). In intact mice$GdCl_3$ did not modify the serum TIMP-1 concentration (Fig. 3). Injection of $GdCl_3$ to mice with LLC led to a significant elevation of serum TIMP-1 concentration in comparison with that in intact animals during all periods of the experiment (Fig. 3). However, injection of $GdCl_3$ to mice with tumors led to reduction of serum TIMP-1 concentration during all periods of the tumor development (except day 11) in comparison with animals with LLC, the levels not reaching those in intact mice (Fig. 3). Similar results were observed in experiments with $GdCl_3$ in doses causing selective blocking of liver and lung macrophages (Fig. 3).

We previously showed that injection of $GdCl_3$ in a dose of 14 or 28 mg/kg to mice with intramuscularly transplanted LLC on days 3 or 8 after tumor transplantation reduced the mean number of tumor metastases in the lungs [1,2]. A more pronounced antimetastatic effect was observed after $GdCl_3$ injection at the stage of tumor dissemination (day 8) [1,2]. Presumably, the positive antimetastatic effect of $GdCl_3$ is explained by

TABLE 1. Serum Concentration of MMP-2 (Active Form+ Proenzyme) in Mice with LLC (*M*±*m*)

Group	MMP-2 concentration, ng/ml
Intact	201.10±4.64
$GdCl_3$	181.70±4.64*
LLC on day 13 after transplantation	236.30±10.23*
LLC on day 13+$GdCl_3$ (14 mg/kg)	189.10±12.91$^+$
LLC on day 17 after transplantation	220.00±34.16
LLC on day 17+$GdCl_3$ (14 mg/kg)	185.70±5.48*

Note. Each group consisted of 7 animals. $p<0.05$ compared to: *intact group, $^+$mice with LLC on day 13 after tumor transplantation.

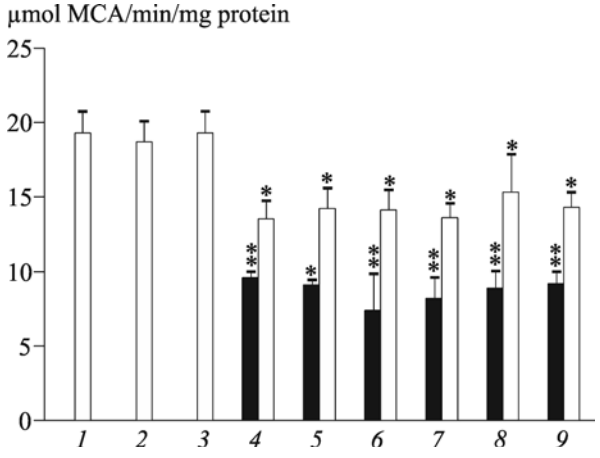

Fig. 2. Activity of MMP in tumor tissue (dark bars) of mice with LLC and in lung tissue (light bars) with metastases. *1*) intact; *2*) intact+$GdCl_3$ (14 mg/kg); *3*) intact+$GdCl_3$ (28 mg/kg); *4*) LLC on day 13 after transplantation; *5*) LLC on day 13+$GdCl_3$; *6*) LLC on day 17 after transplantation; *7*) LLC on day 17+$GdCl_3$; *8*) LLC on day 20 after transplantation; *9*) LLC on day 20+$GdCl_3$. *$p<0.05$, **$p<0.01$ compared to the corresponding parameter in intact mouse lung tissue.

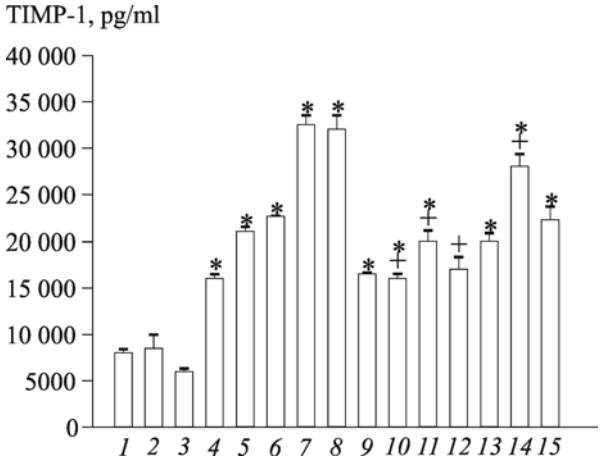

Fig. 3. Serum concentration of TIMP-1. *1*) intact; *2*) intact+$GdCl_3$ (14 mg/kg); *3*) intact+$GdCl_3$ (28 mg/kg); *4*) LLC on day 11 after transplantation; *5*) LLC on day 13; *6*) LLC on day 15; *7*) LLC on day 17; *8*) LLC on day 20; *9*) LLC on day 11 after transplantation+$GdCl_3$ (14 mg/kg); *10*) LLC on day 13+$GdCl_3$ (14 mg/kg); *11*) LLC on day 15+$GdCl_3$ (14 mg/kg); *12*) LLC on day 15+$GdCl_3$ (28 mg/kg); *13*) LLC on day 17+$GdCl_3$ (14 mg/kg); *14*) LLC on day 20+$GdCl_3$ (14 mg/kg); *15*) LLC on day 20+$GdCl_3$ (28 mg/kg). *$p<0.01$ compared to intact mice, $^+p<0.05$ compared to analogous groups receiving no $GdCl_3$.

macrophage depression, changed MMP/TIMP proportion, and disorders in extracellular matrix degradation.

Hence, the increase of serum TIMP-1 concentration is characteristic of LLC progress in mice, while total MMP activity in LLC tissue is reduced presumably because of predominating inert MMP proforms. These data indicate pronounced disorders in MMP regulation by TIMP-1, paralleled by changes in the

system of membrane-bound MMP and proteases located on tumor cell surface. It is noteworthy that injection of $GdCl_3$, causing in various doses selective depression of liver macrophages or of liver and lung macrophage pool, just partially prevented the increase in serum TIMP-1 concentration during LLC development (Fig. 3). High level of TIMP-1 was observed during metastatic development of LLC (after day 7) associated with disorders in the MMP/TIMP-1 proportion and remodeling of extracellular matrix components.

Of endogenous inhibitors of MMP (TIMP-1, -2, -3, -4) known by the present time, TIMP-1 is studied best of all [8-12]. On the one hand, TIMP-1 inhibits invasion and metastasizing of some tumor types [6-8], on the other, it stimulates the growth of some others [8]. High expression of TIMP-1 in human breast cancer at the early stage of the disease is regarded as a predictor of favorable outcome. At later stages of breast cancer high expression of TIMP-1 is associated with rapid progress of the tumor and its metastasizing [6-8]. It was found that TIMP-1 inhibits apoptosis of tumor cell, and hence, its high expression at later stages of tumor development seems to indicate an unfavorable prognosis. The data of this study indicate that high concentration of TIMP-1 in the sera of mice with LLC indicates a pleiotropic pro-tumor significance of TIMP-1 reflecting disorders in MMP regulation during tumor growth and metastasizing.

The authors are grateful to V. I. Kaledin, leading scientist of Institute of Cytology and Genetics, for assistance in experiments and to junior scientist Ya. A. Kisarova for assistance in presentation of the results.

REFERENCES

1. S. Ya. Zhanaeva, T. V. Alekseenko, and T. A. Korolenko, *Byull. Eksp. Biol. Med.*, **146**, No. 11, 548-551 (2008).
2. T. A. Korolenko, T. G. Filatova, N. G. Savchenko, *et al.*, *Ibid.*, **143**, No. 3, 280-284 (2007).
3. K. Almholt, A. Juncker-Jensen, O. D. Laerum, *et al.*, *Mol. Cancer Ther.*, **7**, No. 9, 2758-2767 (2008).
4. K. Brand, *Curr. Gene Ther.*, **2**, No. 2, 255-271 (2002).
5. M. L. Davidsen, S. O. Wurtz, M. U. Romer, *et al.*, *Br. J. Cancer*, **95**, No. 8, 1114-1120 (2006).
6. C. A. Kontogiorgis, P. Papaioannou, and D. J. Hadjipavlou-Litina, *Curr. Med. Chem.*, **12**, No. 3, 339-355 (2005).
7. H. Nagase, R. Visse, and G. Murphy, *Cardiovasc. Res.*, **69**, No. 3, 562-573 (2006).
8. C. M. Overall and C. Lopez-Otin, *Nat. Rev. Cancer*, **2**, No. 9, 657-672 (2002).
9. M. J. Sierovogel, G. Pasterkamp, D. P. de Kleijn, and B. H. Strauss, *Curr. Pharm. Res.*, **9**, No. 13, 1033-1040 (2003).
10. M. D. Sternlicht and Z. Werb, *Annu. Rev. Cell Dev.*, **17**, 463-516 (2001).
11. M. Van Roy, B. Wielockx, A. Baker, and C. Libert, *Cancer Gene Ther.*, **14**, No. 4, 372-379 (2007).
12. R. Visse and H. Nagase, *Circ. Res.*, **92**, No. 8, 827-839 (2003).
13. F. J. Vizoso, L. O. Gonzalez, M. D. Corte, *et al.*, *Br. J. Cancer*, **96**, No. 6, 903-911 (2007).
14. S. O. Wurtz, A. S. Schrohl, N. M. Sorensen, *et al.*, *Endocr. Relat. Cancer*, **12**, No. 2, 215-227 (2005).
15. N. Yukawa, T. Yoshikawa, M. Akaike, *et al.*, *Oncology*, **72**, Nos. 3-4, 205-208 (2007).