ONCOLOGY

Activity and Concentration of Cathepsin B as Prognostic Criteria for the Development of Mouse LS Lymphosarcoma and Lewis Lung Adenocarcinoma

T. A. Korolenko, S. Ya. Zhyanaeva, O. N. Poteryaeva, O. V. Falameeva*, O. A. Levina*, V. I. Kaledin*, and I. Shandula**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 4, pp. 452-455, April, 2002
Original article submitted December 19, 2001

We measured activity and content of cathepsin B in tumor tissues, liver, and spleen in mice with Lewis adenocarcinoma and LS-lymphosarcoma. Cathepsin B activity in Lewis adenocarcinoma cells was lower than in LS-lymphosarcoma cells, which was probably related to differences in their metastatic properties. Antitumor therapy increased activity and content of cathepsin B in tumor tissues. Changes in the content and activity of cathepsin B in tumor tissues can serve as a prognostic criterion for tumor regression during therapy. Cathepsin B is probably involved in apoptosis of tumor cells during chemotherapy of lymphosarcoma-LS with cyclophosphamide.

Key Words: *cathepsin B; activity and concentration; enzyme immunoassay; LS-lymphosarcoma; Lewis lung adenocarcinoma*

Cathepsin B (CB) cleaves synthetic and protein substrates at pH 5.0-8.0 [3]. Similarly to other lysosomal cysteine proteases (cathepsins H, L, and S), CB is in active in weak-acid media under physiological conditions [3,6]. CB is released from lysosomes into the extracellular space with a higher pH value and undergoes inactivation or proteolysis, which accompanies activation of other proteases (*e. g.*, pro-collagenase) during tumor cell invasion [4,6,9,10]. Secretion of cathepsins B and L by tumor cells causes destruction of the extracellular matrix (collagen, elastin, and proteoglycans) and lysis of cell basal membranes, which contributes to invasion of tumor cells [5]. The increase in CB content in the cytosol of tumor cells is associated with high risk of recurrence of breast cancer without lymph node metastases after surgery [10]. High plasma CB concentration is an indicator of poor prognosis in tumors and low life span of patients with tumors of the head and neck [9,13-15].

The aim of the present study was to determine whether the content and activity of CB can serve as prognostic criteria for the development of Lewis lung adenocarcinoma (LLA) and lymphosarcoma-LS in mice receiving effective antitumor therapy.

MATERIALS AND METHODS

LLA was produced in 4-month-old male (CBA×C57Bl)F_1 mice weighing 26-30 g and obtained from the Institute of Pharmacology. Tumor cells were transplanted into right thigh muscles. Cyclophosphamide (CP) was injected intraperitoneally in a single dose of 150 mg/kg [1].

Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences; *Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk; **Institute of Chemistry, Slovak Academy of Sciences, Bratislava. ***Address for correspondence:*** T.A.Korolenko@iph.ma.ru. Korolenko T. A.

0007-4888/02/1334-0392$27.00

TABLE 1. Content and Activity of CB in the Tumor Tissue in Mice with LLA Receiving CP and CMG ($M \pm m$, n=5-10)

Parameter	Control	CP	CMG	CP+CMG
CB, nmol/g protein	0.032±0.004	0.068±0018	0.054±0.005**	0.055±0.004**
CB activity, nmol MCA/mg protein/min	0.108±0.021	0.181±0.031	0.219±0.026**	0.406±0.070*

Note. Here and in Table 2: *p<0.01 and **p<0.05 compared to untreated animals. Here and in Tables 2 and 3: CB activity inhibited by CA-074.

Lymphosarcoma-LS was produced in 2-3-month-old male CBA mice obtained from the Institute of Cytology and Genetics. The animals were intraperitoneally treated with CP and/or β-1,3-D-carboxymethylglucan (CMG, Institute of Chemistry, Slovak Academy of Sciences) in single doses of 50 and 25 mg/kg, respectively [2]. Our previous experiments demonstrated a positive effect of CP alone and in combination with CMG during the therapy of lymphosarcoma-LS [1] and LLA in mice [2], respectively. CP in a dose of 50 mg/kg increased the life span of mice with lymphosarcoma-LS [8].

The animals were decapitated, and the tumor tissue, liver, and spleen were taken. The hindlimbs were cut off. Tumor weight was calculated as the difference between the weights of damaged and intact hindlimbs.

The content and activity of CB were measured in the tumor tissue and in liver and spleen homogenates. Tissue homogenates were prepared in 0.25 M sucrose and 0.001 M ethylenediamine tetraacetate (pH 7.2-7.4). To estimate the content and activity of CB, the samples were treated with Triton X-100 in a final concentration of 0.1%.

CB activity was measured fluorometrically using the substrate Z-Arg-Arg-MCA (Vektor) and selective CB inhibitor CA-074 (10^{-7} M) [3]. The results were expressed in nmol methyl-coumarinyl-amide (MCA)/mg protein/min.

CB content was measured using enzyme immunoassay kits for human CB (KRKA). We found that mouse CB cross-reacts with antibodies against human CB. Therefore, these kits can be used for the measurements of CB in mice.

Calibration solutions (100 ml) with known concentrations of CB were added to polyclonal antibodies against CB in a 96-well plate. After incubation and washing, we added 100 ml monoclonal mouse antibodies against CB labeled with horseradish peroxidase. Enzyme immunoassay was performed by the sandwich technique using horseradish peroxidase as an indicator enzyme. The reaction was visualized with 3,3',5,5'-tetramethylbenzidine. The amount of colored reaction products was measured on a Star 30 Plate Reader multichannel spectrophotometer (Kenstar) at 450 nm. CB content in tissue homogenates was expressed in nmol/g protein.

The results were analyzed by Student's t test (Statistica software). The differences were significant at p<0.05.

RESULTS

CB activity in LLA cells was lower than in LS-lymphosarcoma cells (Tables 1 and 2), which was probably related to differences in their metastatic properties. During metastasizing CB is involved in degradation of the basal membrane, type IV collagen, and laminin and contributes to cell invasion [4,6,16]. The selective CB inhibitor CA-074 completely inactivated the enzyme in the studied tissues. Our results indicate that CB, but not other proteases, is involved in this process. In intact (CBA×C57Bl)F_1 mice CB activities in the spleen and liver were similar (Table 3).

In animals with LLA and lymphosarcoma-LS CB concentrations in tumor tissue were similar (Tables 1 and 2). In intact mice CB content in the liver was comparable with that in the spleen, while in mice with tumors CB concentration in the liver was higher than in the spleen (Table 3).

Combination therapy with CP and CMG, as well as CMG alone (to a lesser extent), increased activity and concentration of CB in tumor tissue in mice with LLA (compared to untreated animals, Table 1).

CP increased the content and activity of CB in tumor tissue in mice with lymphosarcoma-LS (Table 2).

TABLE 2. Content and Activity of CB in the Tumor Tissue and Organs in Mice with Lymphosarcoma-LS Receiving CP ($M \pm m$, n=5-9)

Parameter	Control	CP
CB, nmol/g protein		
tumor tissue	0.049±0.004	0.107±0.028**
liver	0.121±0.017	0.166±0.043
spleen	0.028±0.005	0.185±0.060*
CB activity, nmol MCA/mg protein/min		
tumor tissue	0.220±0.029	0.820±0.125*
liver	0.680±0.031	0.58±0.12
spleen	0.51±0.09	0.88±0.36

TABLE 3. Content and Activity of CB in the Liver and Spleen in (CBA×C57Bl)F_1 Mice with LLA Receiving CP ($M\pm m$, n=4-9)

Parameter	Intact	Control	CP
CB, nmol/g protein			
liver	0.091±0.007	0.162±0.017*	0.179±0.017
spleen	0.081±0.017	0.068±0.015	0.068±0.018
CB activity, nmol MCA/mg protein/min			
liver	0.528±0.090	0.491±0.010	0.578±0.120
spleen	0.901±0.170	1.15±0.10	—

Note. *p<0.05 compared to intact animals.

Liver CB concentration in mice with LLA was higher than in intact animals. However, enzyme activity in these mice did not differ from normal (Table 3). CP did not change CB content in the liver and spleen (compared to untreated animals, Table 3).

In mice with lymphosarcoma-LS receiving CP CB content in the spleen was higher than in untreated animals. However, in these mice enzyme activity in the spleen remained unchanged (Table 2).

Progression of most human tumors, including cancers of the mammary gland, intestine, esophagus, and liver, is associated with increased expression of cysteine (CB), aspartyl (cathepsin D), and serine proteases and metalloproteases (gelatinases A and B) in tumor tissue [7]. The increase in plasma CB level serves as a prognostic criterion for the development of tumors and determines the life span of patients with tumors of the head and neck [9,10].

The intensity of mRNA expression and the content and activity of CB increase in many human tumor cells. A positive correlation was found between malignancy and CB expression, content, and activity [7]. Previous studies showed that cysteine protease inhibitor E-64 and CB pro-peptide block invasion of LLA cells in mice. Moreover, inhibition of CB decreases metastatic activity of tumor cells [7].

CB is localized in lysosomes (perinuclearly) or on the plasma membrane of tumor cells [9]. During malignant transformation CB bound to cell membranes [11] can be secreted intra- or extracellularly [16]. It was hypothesized that CB plays a dual role in the tumor process. On the one hand, CB is involved in apoptosis of tumor cells (positive antitumor effect). On the other hand, this enzyme accelerates tumor invasion and formation of metastases (adverse effect) [5,6]. Various CB activities in LLA and lymphosarcoma-LS cells are probably related to differences in their metastatic properties. Previous studies on cells with various capacities for malignant transformation produced similar results [12].

Effective antitumor therapy of mice with CP produced a similar increase in CB activity, which is considered as a prognostic criterion for malignant transformation [9]. CB content in tumor tissues increased to a lesser extent. Activity and content of CB in the liver and spleen tended to increase in mice with LLA and LS-lymphosarcoma, which is associated with the presence of tumors (Tables 2 and 3).

Our results show that CB activity more precisely reflects the positive effect of antitumor therapy in mice than enzyme content. Therefore, activity of CB can be used as a prognostic criterion for tumor development that reflects the efficiency of antitumor therapy.

Activity and concentration of CB in tumor tissues underwent various changes in mice and humans. Antitumor drugs (especially CP) increase CB activity in mice, but normalize high enzyme activity in humans with breast cancer. CP increased CB activity in mice with lymphosarcoma-LS, which probably reflects apoptosis in tumor cells [8]. Our previous studies showed that CP in a dose of 50 mg/kg causes lymphosarcoma-LS regression in most animals [1]. Tumor development is accompanied by an imbalance between cysteine proteases and their inhibitors due to a sharp decrease in the content of cystatin C and stefin A; these parameters return to normal after the therapy [1,2]. The role of high CB activity in adenocarcinoma remains unclear. The concentration of cysteine protease inhibitors and content and activity of cysteine proteases (*e. g.*, CB) should be used as prognostic factors for tumor regression during effective antitumor therapy.

We thank the KRKA Company that provided enzyme immunoassay kits for measuring CB content. We are grateful to Prof. N. Katunuma for the specific CB inhibitor CA-074.

REFERENCES

1. O. N. Poteryaeva, O. V. Falameeva, S. Ya. Djanayeva, *et al.*, *Byull. Eksp. Biol. Med.*, **132**, No. 7, 77-79 (2001).
2. O. V. Falameeva, O. N. Poteryaeva, S. Ya. Djanayeva, *et al.*, *Ibid.*, **132**, No. 8, 205-208 (2001).

3. A. J. Barrett and H. Kirschke, *Methods Enzymol.*, **80**, 535-561 (1981).
4. A. Donchin, J. I. Suzuki, H. Seki, *et al.*, *Cancer*, **89**, 482-487 (2000).
5. L. Foghsgaard, D. Wissing, D. Mauch, *et al.*, *J. Cell Biol.*, **153**, 999-1010 (2001).
6. B. A. Frosch, I. Berquin, M. R. Emmert-Buck, *et al.*, *APMIS*, **107**, 28-37 (1999).
7. J. T. Koblinski and B. F. Sloane, *Medical Aspects of Proteases and Protease Inhibitors*, Eds. N. Katunuma *et al.*, Amsterdam (1997), pp. 185-194.
8. T. A. Korolenko, O. N. Poteryaeva, S. J. Djanayeva, *et al.*, *Drugs Exp. Clin. Res.*, **26**, No. 5-6, 285-292 (2000).
9. J. Kos, B. Werle, T. Lah, and N. Brunner, *Int. J. Biol. Markers*, **15**, No. 1, 84-89 (2000).
10. T. T. Lah, M. Cercek, A. Blejec, *et al.*, *Clin. Can. Res.*, **6**, 578-584 (2000).
11. K. Moin, L. Cao, N. A. Day, *et al.*, *Biol. Chem.*, **379**, 1093-1099 (1998).
12. C. L. Monito, D. Berkowitz, K. M. Lee, *et al.*, *Am. J. Physiol. Endocrinol. Metab.*, **281**, 289-297 (2000).
13. O. N. Poteryaeva, O. V. Falameyeva, T. A. Korolenko, *et al.*, *Drugs Exp. Clin. Res.*, **26**, No. 5-6, 137-142 (2000).
14. A. A. Sinha, B. J. Quast, M. J. Wilson, *et al.*, *Prostate*, **48**, 274-284 (2001).
15. T. Strojnik, T. Zajc, A. Bervar, *et al.*, *Pflugers Arch.*, **439**, R122-R123 (2000).
16. A. M. Szpaderska and A. Frankfater, *Cancer Res.*, **61**, 3493-3500 (2001).