Proteasome Activity and Their Subunit Composition in Endometrial Cancer Tissue: Correlations with Clinical Morphological Parameters

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The development of endometrial cancer is related to the status of the intracellular proteasome system. Total proteasome activity and pools 26S and 20S activities are higher in tumor tissue than in intact endometrium, and their composition is different. The expression of α1α2α3α5α6α7 is lower in endometrial cancer tissue in comparison with intact endometrium and the content of immune subunits LMP7, LMP2, and PA28β is increased. Total proteasome activity depends on the disease stage.

Key Words: *endometrial cancer; proteasome activity; subunit composition*

Proteasomes characterized by trypsin-like, chymotrypsin-like, and caspase-like activities are highly important for intracellular protein degradation and hence, play an important role in the pathogenesis of many diseases, including their contribution to emergence and development of malignant tumors [11]. Proteasomes are presented by two pools of 26S and 20S. 26S pool specifically degrades proteins and peptides in the cell, while pool 20S destroys abnormal and short-living peptides [2,9]. The subunit composition of proteasomes is also very important. Carcinogenesis is associated with substitution of constitutive Y and X subunits for immune LMP2 and LMP7 and changes in activity of multicatalytic enzymatic complex [8,4].

We previously demonstrated changes in total proteasome activity during the development of endometrial cancer [3]. In addition, experimental studies have demonstrated apoptotic death of endometrial cancer cells under conditions of bortezomib (proteasome inhibitor) treatment [7]. However, the data on the activities of pools 26S and 20S and their subunit composition in endometrial cancer are scanty.

We compared proteasome activities and subunit composition in tumors and histologically intact endometrial tissue and evaluated correlations between these parameters and clinical picture of the disease and tumor morphology.

MATERIALS AND METHODS

The study was carried out in patients with morphologically verified endometrial cancer, stages I-II (*N*=49, mean age 56.8±1.5 years). Of these, 13 patients presented with stage Ia, 24 with stage Ib, 3 with stage Ic, and 9 with stage II disease. The volumes of diagnostic studies and therapy were in line with algorithms of diagnosis and therapy of malignant tumors approved by the Ministry of Health and Social Development of the Russian Federation and included surgical intervention at stage 1 and radiotherapy at stage 2.

Specimens of tumor and histologically normal tissue collected at a distance of at least 1 cm from tumor borderline were studied. The specimens were frozen directly after collection and stored at -80°C.

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For preparing clear homogenate, frozen tissue (100 mg) was homogenized in liquid nitrogen and resuspended in 300 μl 50 mM Tris-HCl buffer (pH 7.5) with 2 mM ATP, 5 mM magnesium chloride, 1 mM dithiotreitol, 1 mM EDTA, and 100 mM sodium chloride. The homogenate was centrifuged for 60 min at 10,000*g* and 4°C.

For proteasome fractionation, all procedures were carried out at 4°C. Proteins of clarified homogenates were fractionated by ammonium sulfate in two stages. The 26S proteasome-rich fraction was obtained by adding ammonium sulfate to 40% saturation, 20S proteasome fraction was obtained by adding ammonium sulfate to 70% saturation [1].

For evaluation of proteasome activity, chymotrypsin-like activity of pooled proteasomes (including 26S and 20S) was measured in clarified homogenates of the tumor and intact tissues by hydrolysis of fluorogenic oligopeptide Suc-LLVY-AMC utilized by chymotrypsin-like proteasome centers [6]. The reaction mixture for evaluation of activities of total proteasome pool and 26S proteasome pool contained 20 mM Tris-HCl (pH 7.5), 1 mM dithiotreitol, 30 μM Suc-LLVY-AMC, 5 mM $MgCl_2$, and 1 mM ATP. The composition of the reaction mixture for measurement of 20S proteasome pool was the same, except for $MgCl_2$ and ATP. The reaction was carried out at 37°C for 20 min. The resultant product was registered on a Hitachi-850 fluorometer at stimulation λ=380 nm and emission λ=440 nm. The quantity of the enzyme hydrolyzing 1 nmol Suc-LLVY-AMC over 1 min was taken as proteasome activity unit. Specific activity of proteasomes was expressed in units of activity per mg protein. Protein was measured by Lowry's method.

Electrophoresis was carried out after Laemmly in 13% PAAG. The samples were applied in a buffer containing 0.0625 M Tris-HCl (pH 6.8), 2% dodecylsulfate, 5% 2-mercaptoethanol, 10% glycerol, and 0.01% bromophenol blue.

After electrophoresis of clarified homogenate proteins in 13% PAAG with sodium dodecylsulfate the polypeptides were transferred onto a Hybond-ECL nitrocellulose membrane (Amersham). The membrane was incubated for 2 h at 20°C in TNT buffer (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% Twin-20) and then in the same buffer with 5% degreased milk and monoclonal antibodies to subunits α1α2α3α5α6α7 or LMP7 or Rpt6, or polyclonal antibodies to proteasome subunits LMP2 and PA28β (1:2500), washed several times in TNT buffer, and incubated (1 h) in TNT buffer with 5% degreased milk and antibodies to peroxidase-conjugated mouse or rabbit IgG (1:10,000). After washout, the membrane was processed routinely using the system for chemiluminescent detection of proteins (Amersham). Band density was evaluated by Image J standard software. The results were expressed in percent of proteasome subunit content in intact tissue (100%).

The results were statistically processed by Statistica 6.0 software. The significance of differences was evaluated by Mann–Whitney's nonparametrical test.

RESULTS

Proteasome activity in endometrial cancer tissue was 2.4 times higher than in intact tissue ($164.1\pm25.6\times10^3$ and $76.1\pm5.0\times10^3$ U/mg protein, respectively). Activity of proteasome 26S pool was by 1.9 times higher than in intact tissue, of 20S pool by 1.4 times higher (Fig. 1). These data attest to more intense intracellular proteolysis resultant from higher activities of all proteasome pools in endometrial cancer tissue in comparison with intact endometrium. More active degradation of proteins in malignant transformation is related to intensive proliferation of cancer cells and apoptosis blocking [10].

Changes in proteasome system activity are explained by its subunit composition [5]. The content of subunits α1α2α3α5α6α7, Rpt6, PA28β and immune subunits LMP2 and LMP7 was studied by Western blotting with specific antibodies (Fig. 2). Study of the subunit composition of proteasomes in endometrial cancer tissue showed a 24.1% reduction of α1α2α3α5α6α7 proteasome subunits in tumor tissue in comparison with intact tissue; the level of LMP7 proteasome immune subunit increased by 49%, of LMP2 by 88%, of PA28β by 29.7%, and of proteasome Rpt6 ATP-dependent subunit increased by 33.7%. The appearance of immune subunits in proteasomes leads to increase of their activity [4]. Presumably, increased content of immune subunit in proteasomes in our study was associated with activation of proteasomes and

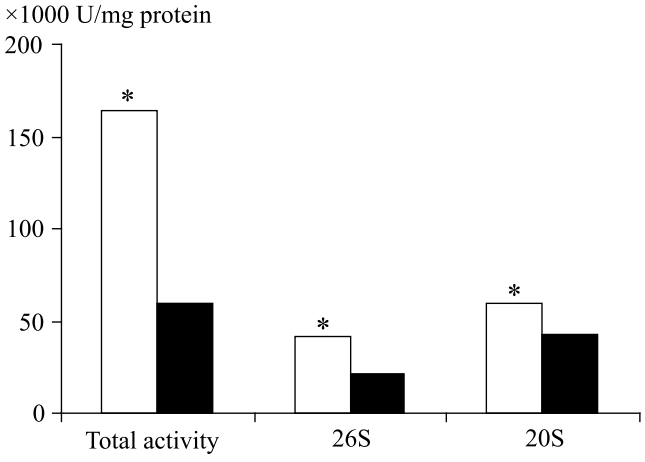

Fig. 1. Total proteasome activity and activities of 26S and 20S pools in endometrial cancer tissue (light bars) and intact tissue (dark bars). *$p<0.05$ *vs.* intact tissue.

TABLE 1. Correlations between Proteasome Activities and Subunit Composition in Endometrial Cancer Tissue and Disease Clinical Picture and Morphology (*M±m*)

Clinical morphological parameters	N	Proteasome activity in tumor tissue, $\times 10^3$ U/mg protein			Proteasome subunit activities in tumor tissue, % of that in intact tissue				
		total	26S	20S	α1α2α3α5α6α7	LMP7	LMP2	PA28β	Rpt6
Disease stage									
Ia	13	110.2±30.3	31.8±7.2	59.8±9.5	78.2±9.7	146.4±13.5	133.4±15.0	133.4±15.0	117.8±13.9
Ib	24	173.5±43.5	49.4±11.8	54.1±11.5	79.8±6.8	157.2±28.2	168.2±33.8	137.5±34.5	144.8±24.5
Ic	3	284.2±80.6	50.0±22.8	74.5±3.2	89.3±30.4	146.6±19.9	262.4±74.5	126.0±1.6	101.1±12.4
II	9	176.6±38.8*	30.6±4.0	66.6±21.9	61.6±21.9	144.3±12.0	158.9±34.1	113.2±13.9	149.2±27.5
Differentiation degree									
low differentiated	6	139.3±32.4	30.9±12.3	76.1±13.9	69.7±10.9	170.4±18.7	151.4±47.4	169.8±52.7	135.2±30.0
moderately ifferentiated	31	182.0±38.0	46.7±9.4	58.6±10.7	76.9±7.1	149.8±17.3	220.6±28.5	124.9±10.2	140.4±19.4
well-differentiated	11	128.1±35.5	33.7±7.5	55.3±11.0	78.5±9.7	137.2±18.1	138.3±21.9	115.5±11.5	117.8±13.5

Note. *$p<0.05$ in comparison with stage Ia.

Fig. 2. Expression of proteasome subunits in the tumor (*1*) and intact tissue in endometrial cancer (*2*). *a*) α1α2α3α5α6α7; *b*) LMP7; *c*) LMP2; *d*) PA28β; *e*) Rpt6.

their pools in tumor tissue in comparison with intact endometrium.

Analysis of correlations of chymotrypsin-like activity of proteasomes in tumors with clinical and morphological parameters of the disease detected an increase in total proteasome activity at later stages of the disease (Table 1). Total proteasome activity was clearly liable to increase (1.5 and 2.5 times) with deeper invasion of endometrial cancer into the myometrium (stages Ib and Ic, respectively, *vs.* stage Ia). Total activity of intracellular enzymes in the group of patients with stage II was 1.6 times higher than in patients with stage Ia. This relationship between proteasome activity and disease stage is practically important and deserves a more ample study. Presumably, the progress of endometrial cancer is associated with more intense intracellular degradation of growth factors, their receptors, and other important intracellular metabolites. No relationship between the studied parameters and tumor differentiation degree was found.

Hence, the development of endometrial cancer is associated with increase of total proteasome activity and activities of pools 26S and 20S. Changes in proteasome activity are related to lower expression of α1α2α3α5α6α7 subunits and higher levels of proteasome subunits LMP2, LMP7, and PA28β. The disease progress is associated with increase of total proteasome activity.

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