ONCOLOGY

Effect of Anthralin on Cell Viability in Human Prostate Adenocarcinoma

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The study revealed the key role of serine protease hepsin activity in transition of *in situ* prostate adenocarcinoma into the metastasizing form. Inhibition of hepsin activity suppresses the invasive growth of the tumor. Hepsin is an convenient target for pharmacological agents, so the study of its inhibitory mechanisms is a promising avenue in drug development. Assay of proteolytic activity in various tumor cell lines *in vitro* showed that this activity in prostate adenocarcinoma cells significantly surpasses proteolytic activity in other examined tumor cell lines. Selective cytotoxic action of anthralin, an inhibitor of hepsin activity, on human adenocarcinoma cells was demonstrated in comparison with other tumor cell lines.

Key Words: *hepsin; proteolytic activity; cytotoxicity; anthralin; prostate adenocarcinoma*

Recent studies attest to importance of the proteolytic system of serine proteases on the cell surface in degradation of extracellular matrix and modulation of cell adhesion during tumor growth and local invasion of the tumor cells [1-7]. This protein family includes also enzyme hepsin located on membranes of prostate epithelial cells in the regions of tight intercellular junctions [4,8]. Hepsin overexpression observed during prostatic adenocarcinoma promotes tumor cell growth accompanied with disorganization of the prostate stroma and degradation of the extracellular matrix components implying involvement of enzymatic activity of hepsin in tumor development [1,6,7,12]. The mechanisms underlying involvement of hepsin into carcinogenesis also deal with modulation of the cell-cell interactions and activation of proteases with their cascades [4,8,10].

Thus, the experimental data indicate important role of proteolytic activity (PA) of hepsin in tumor development. The study of the macromolecular inhibitors of hepsin activity suggests that inhibition of its PA could suppress tumor development. Therefore, this inhibition looks like a promising tool to treat those types of malignant tumors that progress due to activity of this enzyme [9]. Logically, the search for specific inhibitors of hepsin PA is currently in progress to create the corresponding targeted drugs. Anthralin (1,8,9-anthracentriol) is considered as one of the most efficient specific low-molecular inhibitors of hepsin activity that possesses no cytotoxicity towards a number of cell lines [1].

Our aim was to compare PA in tumor cells expressing or not expressing hepsin and to examine anthralin cytotoxicity against human prostatic adenocarcinoma cells.

MATERIALS AND METHODS

LNCaP cells were cultured in DMEM/RPMI-1640 1:1 medium (Thermo Scientific) supplemented with NEAA (Gibco Invitrogen) on poly-L-lysine matrix (Sigma).

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HepG2 cells were cultured in DMEM High medium (Thermo Scientific) supplemented with NEAA. HeLa cells were grown in DMEM High/F12 1:1 medium (Thermo Scientific). All media contained 10% FCS (HyClone), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (Gibco Invitrogen).

The monolayer in wells of a 6-well plate was washed with phosphate buffered saline and incubated at 37°C for 1 h in serum-free DMEM not containing the dye; thereafter the medium was replaced. Then buffer (pH 8.4) containing: 3 M Tris HCl, 0.3 M imidazole, 0.5 M NaCl, and peptide substrate Lys-Pro-Arg-pNA with chromogen group (diacetate paranitroan) responsible for specificity of hepsin (0.19 μM) was added. An aliquot of each sample was transferred to 96-well plate and optical density was measured at λ=415 nm. For measuring PA of lysed cells after washing with phosphate buffered saline, the cells in wells were lysed with RIPA buffer (Pierce) and centrifuged at 3500*g* and 4°C for 10 min. The supernatant was transferred to wells of a 96-well plate and buffer and the substrate (0.19 μM) were added.

Each experimental point was measured in triplicates. Incubation with the substrate was carried out at 37°C for 120 min. PA was measured in the period of 90-120 min and scaled for 10^6 cells.

For MTT assay, the cells were seeded to 96-well plates 6 h before exposure to the preparation (final concentration in wells 10-100 μM). In 72 h, MMT assay was carried out in triplicates.

RESULTS

Bearing in mind that up-regulation of hepsin expression in prostatic tumor cells can results in activation of proteolysis in the pericellular space, we compared PA in monolayer of LNCaP cells (cells of human prostate adenocarcinoma), HepG2 cells (cells of human hepatocellular carcinoma), and HeLa cells (human cervical cancer cells). The experiments showed that the intensity of substrate proteolysis in monolayer of cells of human prostate adenocarcinoma significantly ($p<0.05$) surpassed that in HepG2 and HeLa by 1.7 and 2.8 times, respectively (Fig. 1). The level of PA in the two cell strains not expressing hepsin did not differ significantly. Low level of PA in these cells can be explained by the presence of nonspecific membrane proteases.

It should be noted that PA assay in cell monolayer does not take into account activity of hepsin molecules located at the cell contact sites [4]. As a result, the observed difference in activity between cells expressing and not expressing hepsin could be erroneously diminished. Therefore, lysing the cell membranes increases PA not only due to release of cytoplasmic proteases, but also due to exposure of hepsin molecules

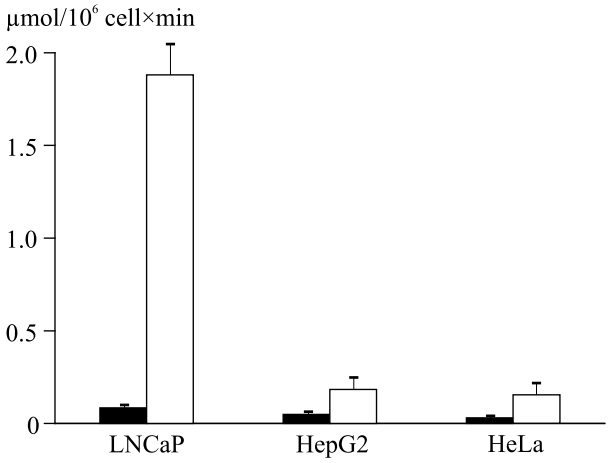

Fig. 1. Proteolytic activity in monolayer (dark bars) and lysed (open bars) LNCaP, HepG2, and HeLa cells.

located in desmosomes. Indeed, a significant increase in PA was observed in lysed LNCaP cells (Fig. 1). PA of lysed HepG2 and HeLa cells also increased in comparison with monolayer, but was considerably lower than PA of LNCaP cells (by 10 and 12 times, respectively, $p<0.05$). The difference between PA of lysed HepG2 and HeLa cell was insignificant. It can be hypothesized that the contribution of cytoplasmic proteases into total PA is insignificant in LNCaP line due to enhanced expression of hepsin and high affinity of this enzyme to the substrate [11]. This view agrees with the data on overexpression of hepsin in prostate adenocarcinoma cells and on its desmosomal locality.

In this study, anthralin demonstrated 10-fold higher toxicity towards LNCaP cells than to HepG2 and HeLa cells (Table 1), while IC_{50} values of vincristine assessed for all three cell strains differed insignificantly indicating specific cytotoxic action of anthralin towards prostate adenocarcinoma cells.

A possible mechanism of high cytotoxicity of anthralin against LNCaP cells can be fatal disturbance of cell-cell contacts eliminating cell communication

TABLE 1. Cytotoxicity of Anthralin and Vincristine for Various Cell Lines

Cell line	IC_{50}, μM	
	anthralin	vincristine
LNCaP	0.63	0.45
HepG2	7.5	0.25
HT1080	21.0	0.04
HEK-93	24.2	0.02
SKOV3	2.5	0.40

essential for cell survival both *in vitro* and *in vivo*. Logically, the targeted action of drugs on hepsin could impair cell-cell contacts, disturb the growth of tumor cells, and/or provoke their death. Due to high specificity of hepsin expression in tumor tissue and its transmembrane locality, this enzyme seems to be an ideal target for drug delivery. Our findings suggest that inhibitors of hepsin PA can be used for this purpose with minimum side effects [13]. The combination of inhibitory and cytotoxic effects of anthralin towards hepsin is worthy of note; this can be used for suppression of tumor invasive potency probably resulting from hepsin PA and for targeted cytostatic action on tumor cells.

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