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Cathepsins and human stomach adenocarcinoma

Katepsyny i gruczolakorak żołądka u człowieka

The malignancy of a tumor has been attributed to the ability of tumor cells to metastasize to secondary sites. During the metastatic cascade a tumor cell passes through several connective tissue barriers. These barriers consist primarily of proteins such as collagen, glycoproteins and proteoglycans. Thus, proteolytic enzymes should facilitate tumor cell invasion and, in turn, metastasis. These enzymes may have their origin in tumor cells or in host cells. Proteolytic activity has been shown to be associated with the plasma membrane of tumor cells as well as to be released from tumor cells. (13, 18).

Other steps in the metastatic cascade which might be mediated by or facilitated by proteolytic enzymes include tumor cell proliferation, angiogenesis, tumor cell detachment from the primary tumor and tumor cell interaction with host cells. The role of proteinases in these processes have frequently been demonstrated indirectly by the use of proteinase inhibitors. The microbial proteinase inhibitors, antipain and leupeptin, inhibit radiation- and chemical-induced carcinogenesis *in vitro* and *in vivo* (6,12). Proteinase inhibitors also decrease the proliferation of tumor cells *in vitro* (10, 28). Proteinases have also been shown to have direct effects on cell-cell adhesion. Proliferation of normal cells *in vitro* can be stimulated by exogenous proteinases (9).

Endopeptidases or proteinases can be subdivided into four classes: serine, metallo, aspartic and cysteine (3, 4). Examples of each class are: plasminogen activator, collagenase, pepsin and cathepsin D. The cysteine proteinase subclass requires activation by thiol reagents.

Cathepsin B has broad specificity as an endopeptidase against protein substrates such as haemoglobin (2), myosin (17), actin (30), troponin (17), tropomyosin (17) and insulin (14), as well as dipeptidylpeptidase activity against glucagon (1) and aldolase (5). Several components of the extracellular matrix, i.e. proteoglycans (15), fibronectin (25) and the nonhelical portion of collagen types I-IV (8), can be degraded by cathepsin B.

Activities of lysosomal enzymes in tumors have been shown to exhibit a correlation with tumor malignancy *in vivo* (33, 34). However, the increased lysosomal enzyme activities in tumors are often attributed to infiltrating host cells or to necrotic tumor cells (9, 34). Sylven and Malmgren (32) reported that the highest lysosomal proteinase activities are associated with the youngest and most rapidly growing tumors. Other authors (31) also reported that lysosomal proteinase activity is highest in the youngest skin carcinomas.

There is an increasing body of literature on release of cathepsin B from human and animal tumors. Thus, the aim of this study is to evaluate the levels of cathepsin B in blood plasma in patients with stomach adenocarcinoma.

MATERIAL AND METHODS

Fifteen human stomach adenocarcinomatous tumors were obtained during surgery. The average age of patients was 57 years. The diagnosis of each case was confirmed histologically in Department of Pathology, Medical Academy in Lublin. Portions of adjacent normal-appearing tissues were obtained for control study.

Tissues were homogenized with a Polytron homogenizer in a 0.1 M phosphate buffer, pH = 6.0 to obtain a 10% homogenate. The homogenate was centrifuged for 15 min at 3. 500 g and in the supernatant the activity of cathepsin B was estimated according to K e i l o v a and T o m a s e k (11). The sample (100 l) was activated for 10 min at 40^0 in 1 ml fo the activating buffer (0.1 M phosphate containing 25 mM cysteine-HCl, 1 mM EDTA, pH = 6.0) The substrate (50 l of a solution of 40 mg of N -benzoyl-D,L-arginine-p-nitro-anilide hydrochloride in 1 ml of dimethylformamide) (Sigma, St. Louis, USA) was then added and the hydrolysis was allowed to proceed at 40 C for 20 min. The hydrolysis of the substrate was terminated by the addition of 100 l of glacial acetic acid. The p-nitroaniline released was determined spectrophotometrically at 405 nm. The quantity of enzyme which liberates 1 mole of p-nitro aniline in 1 min under these conditions was taken as one unit.

Cathepsin H activity was measured according to S c h w a r t z and B a r r e t t (29). Statistical significance of differences was done by computer set Statistica v. 4.0 (Statsoft Inc. Tulsa, USA).

RESULTS

Figure 1 shows cathepsin B and cathepsin H activities in human normal stomach tissue and stomach adenocarcinoma tumors. Activities of both enzymes were significantly elevated in tumor tissue.

DISCUSSION

P o o l e and co-workers (23, 26) have shown that cathepsin B activity in the culture media of explants of malignant human breast tumors is up to eleven times higher than that in the media of explants of normal breast tissue or nonmalignant tumors. Elevated cathepsin B activity has been reported to be present in pancreatic fluid from patients with pancreatic cancer (27), in serum from women with diverse invasive neoplastic diseases (21, 22) including vaginal adenocarcinomas (20) and in urine from women with gynecological cancers (19).

Cathepsin B is apparently released from tumor cells since the activity of this enzyme is present in the media of cultured tumor cells. P i e t r a s and co-workers (22) have found that neoplastic cervical cells release cathepsin B and that the amount of activity released correlates with the rate of cell proliferation *in vitro*.

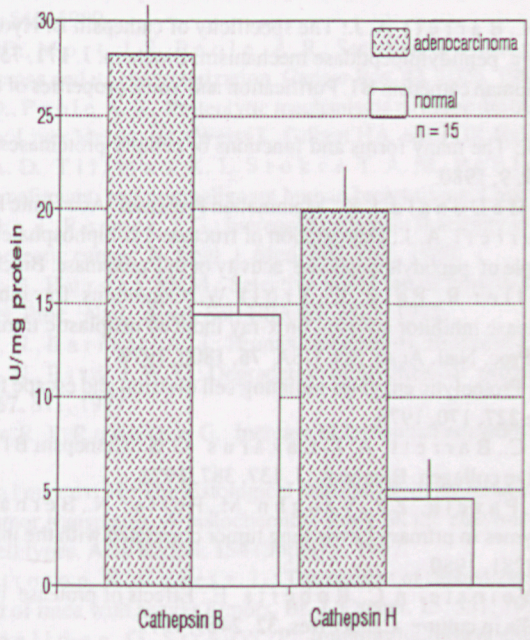

Fig. 1. Cathepsin B and H in stomach tissue. The levels of both cathepsin G and H were significantly lower in normal tisue ($p < 0.01$)

The most suggestive evidence that cathepsin B is also released from tumor cells *in vivo* is that provided by M o r t et al. (16); cathepsin B activity is present in ascites fluid from women with ovarian carcinoma (7). The ascites cells are believed to be the source of the cathepsin B activity since the ascites cells grown in culture release cathepsin B whereas sera from the same patients has no cathepsin B activity. Resident or stimulated peritoneal macrophages are not believed to be the source of activity since murine macrophages in culture do not release cathepsin B into their environment (24, 35).

There is not yet definitive proof that tumor cathepsin B plays a role in tumor invasion and metastasis. However, in a number of animal tumor models there is a correlation between cathepsin B activity and tumor malignancy. This suggest that tumor cathepsin B could be one of the proteinases active in a proteolytic metastatic cascade.

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STRESZCZENIE

Badano aktywność katepsyny B i H w tkance gruczolakoraka żołądka u człowieka. W tkance guzów aktywności tych enzymów były znacznie podwyższone.