ORIGINAL ARTICLE

Lysosomal exoglycosidases and cathepsin D in colon adenocarcinoma

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KEY WORDS

cathepsin D, colon adenocarcinoma, lysosomal exoglycosidases

ABSTRACT

INTRODUCTION Changes in the structure of membrane glycoconjugates and activity of glycosidases and proteases are important in tumor formation.

OBJECTIVES The aim of the study was to compare the specific activity of lysosomal exoglycosidases: N-acetyl-β-D-hexosaminidase (HEX), its isoenzymes A (HEX A) and B (HEX B), β-D-galactosidase (GAL), α-fucosidase (FUC), and α-mannosidase (MAN) with the activity of cathepsin D (CD) in serum, urine, and carcinoma tissue of patients with colon adenocarcinoma.

PATIENTS AND METHODS The specific activity of HEX, HEX A, HEX B, GAL, FUC, MAN, and CD was assayed in serum, urine, and carcinoma tissue of 12 patients with colon adenocarcinoma.

RESULTS Lysosomal exoglycosidases and CD have similar specific activity in colon adenocarcinoma tissue and urine, which is higher than their activity in serum (with the exception of the highest specific activity of CD in urine). A positive correlation was observed between the specific activity of CD and that of HEX, HEX A, FUC, and MAN in the carcinoma tissue and urine as well as between CD and GAL in the urine of patients with colon adenocarcinoma. Negative correlations were observed between protein levels and the specific activity of HEX, HEX A, FUC, MAN, and CD in the carcinoma tissue and urine, and between protein levels and GAL in urine.

CONCLUSIONS Increased degradation and remodeling of glycoconjugates in the colon adenocarcinoma tissue is reflected by increased specific activity of exoglycosidases and CD. The results suggest a strong effect of exoglycosidase action on tissue degradation and a potential role of exoglycosidases in the initiation of proteolysis.

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INTRODUCTION Colon cancer is one of the most common malignant tumors in highly developed European countries. In 2006, there were 3,191,600 cases of cancer in Europe (excluding nonmelanoma skin cancers) and 1,703,000 deaths from cancer. The most common was breast cancer (429,900 cases; 13.5% of all cases), followed by colorectal cancer (412,900; 12.9%), and lung cancer (386,300; 12.1%). Lung cancer, with approximately 334,800 deaths (19.7%), was the most common cause of death from cancer, followed by colorectal (207,400 deaths), breast (131,900), and stomach (118,200) cancer.[1,2]

High incidence of and mortality from colorectal cancer are mainly due to genetic predisposition, coexistence of other diseases, environmental factors, or late detection of cancer. The development of colorectal cancer is a multistep process. Cancer is initially asymptomatic. Therefore, it takes up to several years for small dysplastic foci in the intestinal epithelium to be transformed to the carcinoma in situ.[3] The ability of cancer cells to spread from the primary tumor to lymph nodes, the closest neighborhood and distant tissues and organs, is an inherent feature of malignant tumors and is the main cause of failure in the treatment of many types of cancer.

The basement membrane (BM) is a specialized network of proteins and proteoglycans of the extracellular matrix (ECM), serving as a mechanical barrier between the epithelium (endothelium) and the surrounding tissue. The BM serves as an anchor for cells, which induces their differentiation and limits their migration.[4,5] The ability of carcinoma cells to produce enzymes that degrade the components of the ECM, BM, and tumor stroma is strictly connected with the predisposition to metastasis.[4,5] During metastasis formation, carcinoma cells infiltrate and damage blood vessels by the action of degradative enzymes, which enable the carcinoma cells to enter the circulation. The source of enzymes damaging vessel walls during metastasis could also be lymphocytes accumulated around the tumor due to inflammation. Inflammatory macrophages and neutrophils release elastase, cathepsin G, and lysosomal exoglycosidases to the environment.[6,7] Four classes of proteases take part in the proteolytic degradation of tissues by carcinoma: 1) matrix metalloproteinases, 2) cysteine proteases, 3) aspartyl proteases, e.g., cathepsin D (CD), and 4) serine proteases. Cathepsins are the largest group among proteases.[8,9] The role of particular proteases in the development of tumor is still not fully recognized. However, it is known that tumor invasiveness depends on the activity of proteases bound to the surface of tumor cells.[10] CD (E.C. 3.4.23.5 – enzyme nomenclature) is an aspartyl endopeptidase. CD hydrolyzes peptide bonds, which involve aromatic, mainly hydrophobic amino acids.[8,11] Morever, it participates in the apoptosis by the activation of procaspase 3 and 8.[12,13] CD is an enzyme of proteolytic cascade, which actively participates in the invasion of carcinoma during both local invasion and metastasis formation.[8,10,11]

Changes in the structure of membrane glycoconjugates and in the activity of glycosidases and glycosyltransferases can also be significant in the process of tumor and metastasis formation.[14] It is accepted that endo- and exoglycosidases facilitate destruction of protein core by removing the carbohydrate chains of glycoproteins and proteoglycans and thus allowing access of proteases to otherwise protected regions of the protein.[8]

Lysosomal proteases and exoglycosidases can be released to the cell cytosol, then out of the cell to the blood stream and urine, as a result of increased permeability of lysosomes caused by cancer progression.[15,16] The levels of lysosomal membrane permeability and lysosomal enzymes released into the cell cytosol, blood stream, and urine, reflect the extent of cell damage. Complete disruption of lysosomal membrane leads to uncontrolled death of separate cells and even to tissue necrosis.[8,17]

The following exoglycosidases: N-acetyl--β-D-hexosaminidase (HEX), its isoenzymes A (HEX A) and B (HEX B), β-D-galactosidase (GAL), α-fucosidase (FUC), and α-mannosidase (MAN) release single carbohydrate units from nonreducing ends of oligosaccharide chains[8,18-21] during the catabolism of glycoconjugates (glycoproteins, glycolipids, and glycosaminoglycans) in lysosomes and during the biosynthesis of glycoconjugates in the endoplasmic reticulum and the Golgi apparatus.[8,19] Numerous kidney, liver, and stomach diseases correlate with an increase in the activity of exoglycosidases, especially HEX, in tissues, serum, and urine.[14] The results of previous research[22-25] revealed a significant increase in the activity of lysosomal exoglycosidases: HEX, HEX A and B, GAL, FUC, and MAN in serum and urine as well as CD in the serum, urine, and tissues of patients with colon adenocarcinoma compared with controls.

The aim of the present study was to assess the correlation between the activity of lysosomal exoglycosidases and CD in the carcinoma tissue, serum, and urine of patients with colon adenocarcinoma.

PATIENTS AND METHODS Serum and urine samples were collected before surgery and carcinoma tissue during surgery in the 1st Department of General and Endocrinological Surgery, Medical University of Bialystok, Poland, from 12 patients (7 women and 5 men), aged from 54 to 80 years (mean, 69.83 ±8.86 years), with colon adenocarcinoma, who had not received chemo- and radiotherapy. The study was approved by the local bioethical committee (R-I-003/153/2005 and R-I-003/300/2006). According to the Duke's classification,[26] tumors were of stage G2: not exceeding the wall of intestine (A, n = 4); exceeding the wall of intestine to the serosa or to the perirectal adipose tissue (B, n = 3); with metastases to lymph nodes (C, n = 5).

Blood samples were taken from the cubital vein during the implementation of a catheter to premedication, and urine from the midstream of morning specimen. Serum (after blood clotting) and urine were centrifuged at 4000 × g for 10 minutes at 4°C. Tissue samples were suspended at 0.15 M ice-cold solution of KCl containing 0.2% triton X-100 at the proportion of 1:9 and homogenized in containers immersed in ice. Homogenates were centrifuged at 10,000 × g for 30 minutes at 4°C. Supernatants were stored at –80°C. All tests were run in duplicates. HEX,

TABLE 1 Specific activity of lysosomal exoglycosidases and cathepsin D in serum, urine, and carcinoma tissue of patients with colon adenocarcinoma (n = 12)

	Exoglycosidases, pKat/mg of protein						Cathepsin D, nmol Tyr/mg protein/6 h
	HEX	HEX A	HEX B	GAL	FUC	MAN	
tissue	5281.94 ±2418.82	3373.34 ±1801.34	1876.14 ±1389.26	1025.61 ±426.19	601.51 ±250.13	679.16 ±241.04	170.65 ±41.01
serum	24.523 ±10.854	14.022 ±10.983	10.501 ±4.502	2.901 ±1.356	5.518 ±1.843	3.408 ±1.621	3.638 ±0.522
urine	3600.48 ±2609.96	1992.14 ±1520.53	1608.34 ±1371.66	963.58 ±649.19	500.04 ±156.53	543.03 ±242.00	1242.97 ±875.22
P							
tissue vs. serum	<0.0001	<0.0001	<0.001	<0.0001	<0.0001	<0.0001	<0.0001
serum vs. urine	<0.002	<0.003	<0.005	<0.002	<0.0001	<0.0001	<0.002
tissue vs. urine	NS	0.0744	NS	NS	NS	NS	<0.004

Data are presented as mean ± standard deviation.

Abbreviations: FUC – α-fucosidase, GAL – β-D-galactosidase, HEX – N-acetyl-β-D-hexosaminidase, HEX A – isoenzymes A, HEX B – isoenzymes B, MAN – α-mannosidase, NS – nonsignificant

TABLE 2 Correlations between specific activity of cathepsin D and lysosomal exoglycosidases in tissue, serum, and urine of patients with colon adenocarcinoma (n = 12)

Cathepsin D, nmol Tyr/mg protein/6 h	Exoglycosidases, pKat/mg of protein					
	HEX	HEX A	HEX B	GAL	FUC	MAN
tissue	$r = 0.69$ $P = 0.02$	$r = 0.78$ $P = 0.01$	$r = 0.25$ $P = 0.47$	$r = 0.48$ $P = 0.14$	$r = 0.77$ $P = 0.01$	$r = 0.7$ $P = 0.02$
serum	$r = –0.25$ $P = 0.46$	$r = –0.19$ $P = 0.58$	$r = –0.17$ $P = 0.63$	$r = –0.073$ $P = 0.83$	$r = –0.12$ $P = 0.74$	$r = –0.1$ $P = 0.8$
urine	$r = 0.64$ $P = 0.04$	$r = 0.76$ $P = 0.01$	$r = 0.44$ $P = 0.18$	$r = 0.7$ $P = 0.01$	$r = 0.69$ $P = 0.01$	$r = 0.7$ $P = 0.02$

Abbreviations: see TABLE 1

HEX B, GAL, FUC, and MAN were determined using the method by Marciniak et al.,[27] modified by Szajda et al.[22,23] with 4-nitrophenol derivatives of appropriate sugars as substrates. 4-nitrofenol, released from particular substrates, was measured at 405 nm using a microplate reader EL_x800 and KC junior computer program (Bio-Tek instruments, Winooski, Vermont, United States).

The activity of CD was determined by the Folin-Ciocalteau method[28] as modified by Greczaniuk et al.,[29] and later adapted by Szajda et al.[24]

Total protein was determined using the method by Lowry et al.[30] with lyophilized albumin as standard (Sigma, St. Louis, Missouri, United States).

The statistical pack SPSS®8.0 for Windows PL (SPSS, Chicago, Illinois,Unites States) was used for statistical analysis with the Mann-Whitney *U* test. The Spearman's rank correlation coefficient was used to measure the statistical dependence between 2 variables. The level of *P* less than 0.05 was considered statistically significant.

RESULTS The specific activity of exoglycosidases is similar in colon adenocarcinoma tissue and urine, being significantly higher than in blood serum (TABLE 1). The specific activity of CD is the highest in cancer tissue and the lowest in serum.

Correlations between specific activities of exoglycosidases and CD in the carcinoma tissue, serum, and urine are presented in TABLE 2. In the colon carcinoma tissue, there were strong correlations between specific activity (pKat/mg protein) of HEX ($r = 0.6876$, $P = 0.019$), HEX A ($r = 0.777$, $P = 0.005$), FUC ($r = 0.769$, $P = 0.006$), MAN ($r = 0.699$, $P = 0.017$) and specific activity of CD (nmol Tyr/mg protein/6 h). In the urine of patients with colon adenocarcinoma, there was a strong correlation between specific activity of CD and the specific activity of HEX ($r = 0.636$, $P = 0.035$), HEX A ($r = 0.756$, $P = 0.007$), GAL ($r = 0.697$, $P = 0.008$), FUC ($r = 0.681$, $P = 0.01$), and MAN ($r = 0.618$, $P = 0.024$). TABLE 3 shows a strong negative correlation between protein concentration (mg/ml) and the specific activity of HEX ($r = –0.628$, $P = 0.038$), HEX A ($r = –0.776$, $P = 0.005$), FUC ($r = –0.740$, $P = 0.009$), MAN ($r = –0.605$, $P = 0.048$), and CD ($r = –0.843$, $P = 0.001$) in the carcinoma tissue. We did not find any correlations between the specific activity of exoglycosidases as well as CD and protein in serum. In urine,

TABLE 3 Correlations between protein concentration and specific activity of lysosomal exoglycosidases and cathepsin D in tissue, serum, and urine of patients with colon adenocarcinoma (n = 12)

Protein, ng/ml	Exoglycosidases, pKat/mg of protein						Cathepsin D, nmol Tyr/mg protein/6 h
	HEX	HEX A	HEX B	GAL	FUC	MAN	
tissue	$r = -0.63$ $P = 0.04$	$r = -0.78$ $P = 0.005$	$r = -0.14$ $P = 0.69$	$r = -0.55$ $P = 0.08$	$r = -0.74$ $P = 0.01$	$r = -0.61$ $P = 0.05$	$r = -0.84$ $P = 0.001$
serum	$r = -0.09$ $P = 0.8$	$r = 0.14$ $P = 0.67$	$r = -0.56$ $P = 0.06$	$r = 0.47$ $P = 0.13$	$r = 0.27$ $P = 0.41$	$r = 0.3$ $P = 0.35$	$r = -0.11$ $P = 0.76$
urine	$r = -0.64$ $P = 0.034$	$r = -0.65$ $P = 0.03$	$r = -0.54$ $P = 0.09$	$r = -0.71$ $P = 0.01$	$r = -0.7$ $P = 0.01$	$r = -0.68$ $P = 0.01$	$r = -0.62$ $P = 0.02$

Abbreviations: see **TABLE 1**

we observed negative correlations between protein concentration and specific activity of HEX ($r = -0.638$, $P = 0.034$), HEX A ($r = -0.645$, $P = 0.032$), GAL ($r = -0.708$, $P = 0.007$), FUC ($r = -0.695$, $P = 0.008$), MAN ($r = -0.672$, $P = 0.012$), and CD ($r = -0.613$, $P = 0.024$).

DISCUSSION It is important to detect cancer at an early stage of development to implement appropriate treatment and increase the patient's chances of recovery. Early detection of cancer depends on the progression in oncological diagnostics and studies on tumor markers indicating cancer presence and development.[2,31]

Studies based on lysosomal exoglycosidases describe significantly increased activity of exoglycosidases in the tissue of gliomas[32] and renal carcinoma,[33] as well as in the serum and urine of patients with pancreatic cancer[34,35] and colon adenocarcinoma.[22,23] Increased activity of exoglycosidases in cancers can be explained by their active participation in the degradation and tissue remodeling around the site of carcinogenesis. Recently, there have also been numerous studies on the activity of CD and its significance in cancer diagnostics. A significant increase in the activity of CD was observed in the uterine fibroid tissue,[36] breast,[37,38] cervical,[39] endometrial,[40] prostate,[41] brain,[42] thyroid,[43] laryngeal,[44] liver,[45] gastric,[46] colorectal,[24,47] skin,[48] and head and neck cancers.[49]

TABLE 1 presents similarities in the specific activity of lysosomal exoglycosidases. In the carcinoma tissue and urine, a higher specific activity of lysosomal exoglycosidases was observed compared with serum. CD activity in urine was higher than that in tissue, and was lowest in serum. The lowest specific activity of exoglycosidases and CD observed in serum may be due to the high levels of acute-phase proteins, typically observed in patients with colon adenocarcinoma.[50] In our study, we observed positive correlations between specific activities of HEX, HEX A, FUC, and MAN and CD in the colon cancer tissue and urine, which suggests that HEX and HEX A, FUC, MAN, and CD cooperate in the degradation of the BM, ECM, and cell surface glycoconjugates, oligosaccharide, and polypeptide chains, thus actively participating in the local infiltration and metastasis of cancer. Our study showed a negative correlation between protein and specific activity of lysosomal exoglycosidases as well as CD in the colon cancer tissue and urine, which confirms the involvement of hydrolases in the degradation of proteins and glycoconjugates (glycoproteins, glycolipids, and proteoglycans) of the cancer cells and confirms earlier hypothesis that protein deglycosylation may be a critical initial step leading to the subsequent proteolysis.[17]

It may be stated that the strong correlation between the specific activity of lysosomal exoglycosidases and that of CD in the colon cancer tissue and urine suggests participation of exoglycosidases and CD in the cancerous process and tissue remodeling. Therefore, lysosomal hydrolases (exoglycosidases and CD) had been previously proposed to be cancer markers in cancerous tissues and urine as well as useful parameters in the diagnosis and treatment of colon cancer.[23,27,34,51,52]

An increase in the specific activity of exoglycosidases and CD is therefore most probably involved in the degradation and remodeling of tissue glycoconjugates in the course of colon adenocarcinoma. The negative correlation between protein concentration and the specific activity of exoglycosidases and CD observed in our study as well as the positive correlation between the specific activity of exoglycosidases and CD in the carcinoma tissue and urine indicate that the action of exoglycosidases on tissue degradation by cancer is a vital part of the progression of cancer and highlight the potential role of exoglycosidases in the initiation of proteolysis.

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ARTYKUŁ ORYGINALNY

Egzoglikozydazy lizosomalne i katepsyna D w gruczolakoraku jelita grubego

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SŁOWA KLUCZOWE

egzoglikozydazy lizosomalne, gruczolakorak jelita grubego, katepsyna D

STRESZCZENIE

WPROWADZENIE Zmiany w strukturze glikokoniugatów błonowych oraz w aktywności glikozydaz i proteaz są istotne w rozwoju nowotworu.

CELE Celem badania było porównanie specyficznej aktywności egzoglikozydaz lizosomalnych: N-acetylo-β-D-heksozaminidazy (HEX), jej izoenzymów A (HEX A) i B (HEX B), β-D-galaktozydazy (GAL), α-fukozydazy (FUC) i α-mannozydazy (MAN) z aktywnością katepsyny D (CD) w surowicy, moczu i tkance raka u pacjentów z gruczolakorakiem jelita grubego.

PACJENCI I METODY Aktywność specyficzną HEX, HEX A, HEX B, GAL, FUC oraz MAN oznaczano w surowicy, moczu oraz w tkance guza u 12 chorych z potwierdzonym histopatologicznie gruczolakorakiem jelita grubego.

WYNIKI Egzoglikozydazy lizosomalne i CD mają podobną aktywność specyficzną w tkance gruczolakoraka jelita grubego i w moczu, która jest większa niż w surowicy (z wyjątkiem najwyższej aktywności specyficznej CD w moczu). Stwierdzono dodatnią korelację między aktywnością specyficzną CD oraz HEX, HEX A, FUC i MAN w tkance raka i w moczu oraz między CD i GAL w moczu pacjentów z gruczolakorakiem jelita grubego. Ujemne korelacje stwierdzono między stężeniem białka i aktywnością specyficzną HEX, HEX A, FUC, MAN i CD w tkance raka i moczu oraz między stężeniem białka i GAL w moczu.

WNIOSKI Zwiększona degradacja i przebudowa glikokoniugatów w tkance gruczolakoraka jelita grubego znajdują odzwierciedlenie w zwiększonej specyficznej aktywności egzoglikozydaz i CD. Wyniki sugerują duży wpływ aktywności egzoglikozydaz na degradację tkanek i potencjalną rolę egzoglikozydaz w inicjacji proteolizy.

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