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## Evaluation of serum cathepsin B and D in relation to clinicopathological staging of colorectal cancer

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### Abstract

**AIM:** Proteolytic degradation of the extracellular matrix facilitates cancer invasion and promotes metastasis. The study aims at evaluation of preoperative and postoperative serum cathepsins B and D levels in correlation with selected anatomoclinical features of colorectal cancer.

**METHODS:** Blood samples were collected from 63 colorectal cancer patients before curative operation of the tumor 10 d later. Blood that was obtained from 20 healthy volunteers, served as a control. The activity of cathepsin B was measured with Bz-DL-arginine-pNA as a substrate at pH 6.0, while cathepsin D activity was determined with urea-denatured hemoglobin (pH 4.0).

**RESULTS:** The preoperative and postoperative activities of cathepsin B were significantly ( $P < 0.00001$ ) lower in serum of colorectal cancer patients than in control group. However, postoperative values of this protease were significantly increased in comparison with preoperative ones ( $P = 0.031$ ). Activity of cathepsin D appeared to be significantly higher in colorectal cancer sera ( $P < 0.00001$ ) compared with controls. No statistically significant differences between preoperative and postoperative activity of cathepsin D were noted ( $P = 0.09$ ). We revealed a strong linkage of cathepsins' levels with lymph node status and pT stage of colorectal cancer.

**CONCLUSION:** Blood serum activities of cathepsin B and D depend on the time of sampling, tumor size and lymph node involvement. Significantly, increased activity of cathepsin D could indicate a malignant condition of the large intestine. In our work, the serum postoperative decrease of cathepsin B activity appears as an obvious concomitant of local lymph node metastasis-the well-known clinicopathological feature of poor prognosis.

### INTRODUCTION

Normal cells undergo several changes to converse into invasive malignant clones with metastatic capability. Progress of these alternations is manifested in distinguishable histological and temporal stages-for instance: normal tissue, hyperplasia with a high incidence of proliferating cells, dysplasia with the induction of angiogenesis that precedes following metamorphosis of observed cell clusters into tumors with metastasis. Analysis of the subsequent stages of tumor progression has provided a multistage theory of carcinogenesis on the basis of genetic changes that include activation of oncogenes, inactivation of tumor suppressor genes, and altered expression of tumor-associated molecules.

Cellular transformation provokes tissue remodeling inside neoplastic lesions and in the periphery of the tumor. Disorders in stroma or extracellular matrix (ECM) play an essential role in cancer progression. It is suggested that perturbation of the tissue microenvironment may be sufficient to induce tumor formation. Moreover, local carcinomatous invasion and metastasis also require the destruction of the EMC during local dissemination of malignant cells, angiogenesis, intravasation and extravasation. These processes are enabled by multiple degradation of stromal structure which is achieved due to cooperation of various specific intra- and extracellular proteases. Lysosomal aspartyl and cysteine enzymes-cathepsin D and B are the most important of intracellular proteases that participate in stromal destruction, which facilitates tumor invasion of deeper tissue layers<sup>[1]</sup>. These enzymes can act directly by proteolysis of EMC components or indirectly by costimulation of a cascade of other proteases such as metalloproteases or elastase, which then decompose protein elements of the EMC and basal membranes. This stromal disarrangement allows migration of malignant cells to different regions and compartments of the human body, what accelerates tumor growth and favors metastasis. Not only does overexpression of cathepsin B and D in cancer cells reflect intensity of

neoplasm development, but also increased serum activity of mentioned proteases does the same as well. Apart from mentioned functions, independently of its catalytic activity human cathepsin D stimulates tumor growth as a direct or indirect mitogenic factor for cancer cells<sup>[1]</sup>. Cathepsin D affects tumor angiogenesis in a mysterious way. It was suggested that cathepsin D might favor angiogenesis by releasing ECM-bound bFGF<sup>[2]</sup>. Suggestions of its proangiogenic function contrast with the fact that pro-cathepsin D is responsible for the generation of specific inhibitor of angiogenesis-angiostatin<sup>[3]</sup>. Now it is presumed that cathepsin D may stimulate endothelial cell growth via a paracrine loop, acting as a protein ligand, by directly or indirectly triggering a yet unidentified cell surface receptor<sup>[4]</sup>.

Increased expression, enhanced secretion and cell surface association of cathepsin B were found in different types of tumor cells especially in their more malignant variants<sup>[5]</sup>. Last studies have revealed that augmented production and release of cathepsin B in tumor cells lead to tumor cell growth, invasion and metastasis<sup>[6]</sup>. This cathepsin may act at the contact regions of tumor cells and basement membrane or interstitial stroma. Low pH is necessary for activation of secreted precursors to active forms, which degrade the protein components of basement membranes and interstitial connective matrix including laminin, fibronectin, elastin, and various types of collagen<sup>[7]</sup>. Cathepsin B split fibronectin into smaller parts and change conformation of this protein components. In such a way the CS-1 sequence and alternatively spliced type III connecting segment (IIICS) are uncovered, exposed and recognized by the integrin  $\alpha_4\beta_1$ -receptor<sup>[8]</sup>. Thereby, cathepsin B may be involved in cellular signal transduction beside extracellular protein degradation.

Therefore, the aim of this work was to investigate the activity of lysosomal proteases-cathepsin B and D in blood serum and their potential impact on cancer progression and metastasis in cases of colorectal cancer.

## MATERIALS AND METHODS

The study included 63 patients (42 men and 21 women) with a mean age of 64 years that were required to undergo a colorectal cancer operation. Thirty tumors were located in the rectum and 33 were found in the colon. The patients had no preoperative chemo- or radiotherapy. All the patients were monitored after the operation. Two independent pathologists assessed conventional histopathological parameters (including AJCC/UICC TNM stage, tumor type and grade of differentiation). Differentiation and histological type of the tumors was determined following the World Health Organization guidelines. Fifty-eight colorectal cancers were classified histopathologically as adenocarcinoma and 5 as mucinous adenocarcinoma: 43 cases with G2 grade and 15 cases with G3 grade. Because of too small a number of tumors that were classified as pT1, pT2 and pT4 patients were divided into two groups: five tumors comprised pT1+pT2 group and 53 cancer cases belonged to pT3+pT4 group. Forty-six percent patients had involved lymph nodes at the time of diagnosis.

### Biochemical analysis

The blood samples were collected from colorectal cancer

patients twice: before and 10 d after the curative operation. The blood was centrifuged at 4 °C and the serum samples were stored at -80 °C until examination. The control group included of 20 healthy volunteers (12 men and 8 women) with a mean age of 59 years. The control blood samples were collected only once. Serum was gained from collected blood and the activity of cathepsin B was determined with Bz-DL-arginine-pNA as a substrate at pH 6.0<sup>[9]</sup>, while the cathepsin D activity was measured with urea-denatured hemoglobin (pH 4.0)<sup>[10]</sup>.

## RESULTS

The preoperative and postoperative activities of cathepsin B were significantly ( $P < 0.00001$ ) smaller than in control group. However, postoperative values of this protease were significantly increased in comparison with preoperative ones ( $P = 0.031$ ). On the other hand the activity of aspartyl protease-cathepsin D appeared to be significantly higher ( $P < 0.00001$ ) in comparison with the control group data. In addition, no statistically significant differences between preoperative and postoperative activity were noted ( $P = 0.09$ , Table 1).

**Table 1** Activity of cathepsin B and D in serum of patients with colorectal carcinoma

	Healthy people (n = 20)	Control serum	66.9±2.8
	Colorectal patients (n = 63)	Before operation1 (serum sample a) After operation (serum sample b)	7.9±8.5 <sup>1b</sup> 21.0±8.7 <sup>f</sup>
Cathepsin B nmolPNA/mL			
	Healthy people (n = 20)	Control serum	30.2±2.2
	Colorectal patients (n = 63)	Before operation (serum sample a) After operation (serum sample b)	58.7±24.2 <sup>2d</sup> 49.9±16.9 <sup>b</sup>
Cathepsin D nmol Tyr/mL			

Statistical differences: <sup>1</sup> $P = 0.031$  -comparison between cathepsin B serum samples a and b. <sup>2</sup> $P = 0.09$  -comparison between cathepsin D serum samples a and b. <sup>b</sup> $P < 0.00001$ , <sup>d</sup> $P < 0.00001$ , <sup>b</sup> $P < 0.00001$ , <sup>f</sup> $P < 0.00001$  vs control serum.

We revealed a strong linkage of cathepsins' levels with lymph node status and pT stage of colorectal cancer. As referred to TNM classification, patient group of pT1 and pT2 tumors showed significantly higher preoperative serum activity of cathepsin B than it was detected in preoperative serum of patients with tumors classified to pT3 and pT4 group ( $P = 0.0057$ ). This trend of differences was not apparent enough to reach a level of statistical significance in case of postoperative serum values of cathepsin B. We assume that statistical significance could not be achieved in analysis of homologous postoperative data because of wide variation of postoperative cathepsin B values in set of patients with pT1 and pT2. Similarly values of cathepsin D before operations (serum sample A) were more increased with nearly statistical significance in pT1 and pT2 vs pT3 and pT4 groups respectively ( $P = 0.053$ ).

As shown in Table 2, node-negative (N-) colorectal cancer patients displayed significantly higher postoperative

serum levels of cathepsin B in opposition to patients with lymph node involvement (N+) ( $P = 0.0054$ ). These groups of patients did not significantly differ in cathepsin B levels before operation. We drew out a similar comparison between activities of cathepsin D in node negative and node positive sets of colorectal cancer-but inversely to cathepsin B-remarkable distinction of cathepsin D serum levels appeared before surgery. Namely, the preoperative serum activity of cathepsin D was increased in node negative (N-) *vs* node positive (N+) colorectal cancer patients with statistical significance ( $P = 0.019$ ).

## DISCUSSION

Cancer development is characterized by severe disarrangement of the intra- and extracellular metabolism and structural alterations. Cancer cells overexpress and secrete a large proportion of lysosomal proteases-cathepsins. The activity increase of cathepsin B and D was discovered to be statistically significant in both the neoplastic tissue cytosol and homogenate, compared to the cytosol and homogenate of adjacent healthy tissue<sup>[11]</sup>. Marked tissue immunoreactivity of cathepsin B and D takes place in carcinoma of uterus, ovary, lung, intestines and many other organs<sup>[12-15]</sup>. Moreover, it was reported that tumor cell lines secrete cathepsin B and D<sup>[16]</sup>. On the other hand it was reported that serum cathepsin B level did not differ significantly both in benign and malignant neoplasms of ovaries<sup>[17]</sup>. In cancers there was elevated serum value of cystatin C in comparison with benign ovarian tumors and controls, which negatively affected the amount of cathepsin B. However, ovarian cancer cells are strongly positive for cathepsin B in immunohistochemical analysis, while benign ovarian tumors lacked any positive reaction of this kind<sup>[17]</sup>.

It is suggested that colorectal cancer development is enhanced by oxidative stress. Oxidative stress is maintained as the effect of excessive concentration of lipid hydroperoxides in the vascular net and their participation in the oxidant generation. The other possible explanation might be overproduction of oxidants by inflammatory and/or cancer cells and suppression of antioxidant system in cancer cells. In these conditions free radicals may enhance lipid peroxidation.

Products of this process are responsible for membrane alteration. Reactive oxygen species cause protein damage and as a result changes in biological function of active proteins and membrane permeability for intracellular compounds may appear due to increased susceptibility for drugs that induce apoptosis of neoplastic cells<sup>[1,18]</sup>. It may explain translocation of cathepsins from lysosomes via cytosol and cellular membrane into body fluids.

As we have demonstrated in our previous works, generation of free radicals accompanies the development of cancer<sup>[18]</sup>. Reactive oxygen species cause an oxidative damage of cell membranes which results in an increase of membrane permeability. That implies influx of cathepsin D to extracellular fluid. Therefore, elevated activity of cathepsin D is observed in the serum as an evidence for impairment of general cellular functions like maintenance of the barrier between intracellular and extracellular environment. Advancement of tumor growth and neoplastic spreading across the whole organism seem to reflect the severity of cellular damage. Cathepsin B -cysteine protease is so sensitive to free radicals, that the latter probably inactivate cathepsin B. Thereby it would explain why serum activity of cathepsin B is lower in serum of patients with colorectal cancer in comparison to serum values of this protease in healthy volunteers. Production of free radicals is upregulated and activity of cathepsin B falls down along with the enlargement of tumors and neoplastic extent into lymph nodes. This statement is supported by our present study in which we detected lower levels of cathepsin B in group pT3 and pT4 *vs* pT1 and pT2 as well as in node positive (N+) *vs* node negative (N-) patients after surgery. Total resection of cancer could contribute to limit ROS generation and by means of the inactivation of cathepsin B, ROS generation was reduced enough which is shown in the results obtained.

The concentrations of cathepsin B and D were increased also in the blood serum of patients with other carcinomas<sup>[19-21]</sup>. Additionally, the increase of cathepsin B correlated with the advancement of cancer assessed with Duke's scale. Moreover patients with both elevated levels of cathepsin B and CEA were classified as the group of poor prognosis<sup>[20]</sup>. Nevertheless, in our present investigations, we discovered

**Table 2** Correlation of serum cathepsin B and D activity and clinicopathological findings

Variable		n = 63	Cathepsin B (nmol/L pNA/mL)				Cathepsin D (nmol Tyr/mL)			
			Before operation	P	After operation	P	Before operation	P	After operation	P
Sex	M	42	19.1±8.9	NS	21.7±9.1	NS	59.4±26.3	NS	49.5±16.3	NS
	F	21	15.3±6.6		19.6±7.9		57.0±19.2		50.9±18.4	
Age (yr)	≤60	16	20.4±12.3	NS	17.4±8.0	P = 0.064	54.2±17.0	NS	51.7±22.0	NS
	>60	47	17.02±6.6		22.4±8.7		60.2±26.1		49.3±14.7	
Tumor site	Rectum	30	16.5±7.4	NS	18.4±7.6	NS	59.8±21.6	NS	51.2±19.9	NS
	Colon	33	19.1±9.2		23.5±9.1		57.7±26.3		48.8±13.6	
HPtype	Adc.	58	18.1±8.4	NS	21.02±8.9	NS	58.7±24.4	NS	50.7±17.0	NS
	Adc. m.	5	10.1±9.3		20.9±7.0		56.0±21.2		43.2±14.2	
pT	pT 1+2	5	25.3±0.7	P = 0.0057	30.3±18.7	NS	76.8±23.0	P = 0.053	48.8± 16.5	NS
	pT 3+4	53	17.1±8.5		20.3±7.3		56.7±23.6		50.1± 17.0	
G	2	43	18.8±8.8	NS	21.5±9.2	NS	62.5±25.5	NS	50.4±17.3	NS
	3	15	15.8±7.3		19.9±7.7		50.3±18.8		49.2±16.2	
N	(-)	34	18.5±8.5	NS	23.9±8.9	P = 0.0054	65.9±26.5	P = 0.019	49.7±17.2	NS
	(+)	29	17.03±8.4		17.3±7.1		49.1±16.7		50.3±16.6	

Statistical differences: see Table 1.

only the increase of cathepsin D while levels of cathepsin B were decreased in sera of colorectal cancer patients.

Overexpression of cathepsins may prelude the loss of integrity of ECM protein components. It is known that cathepsin D as endopeptidase degrades ECM proteins and proteins of the basal epithelium as well as many intracellular and endocytosed proteins. It occurs mainly in phagosome-like acid vesicles with acidic pH where EMC components are trapped<sup>[22]</sup>. Cathepsin D may cooperate with cathepsin B in the process of proteolysis and cancer progression. Cathepsin D probably activates cysteine procathepsins B and L<sup>[23]</sup>. Cathepsin B activates the urokinase-type plasminogen which can subsequently activate the plasmin-metalloproteinases proteolytic pathway<sup>[24,25]</sup>. Moreover, cathepsin B may change the balance of metalloproteinases and their inhibitors and directly cleave and inactivate some of MMPs inhibitors -TIMP-1 and TIMP-2<sup>[26]</sup>. In such a way cathepsin B assists tumor cells in their detachment from ECMs and metastasis. Furthermore during proteolysis of interstitial extracellular structure some ECMs-bound growth factors, e.g. bFGF, EGF, TGF- $\beta$ , IGF-I and VEGF, may get free from their chains of connections to the matrix and thanks to the fact that they can escape to join suitable receptors of stromal and tumor cells which indicates their bioavailability for growth modulation<sup>[27]</sup>.

Cathepsin B can multiply the effect of protein digestion and disarrangement by triggering trypsinogen activation<sup>[28]</sup>. Lysosomal cathepsin B activates trypsinogen *in vitro* and the intensity of trypsinogen activation improves with acidic pH<sup>[15,29]</sup>. Human trypsinogen is produced on a wide scale by human colorectal cells<sup>[30]</sup>. Anyway further findings in colon cancer cell lines suggest that this production is limited only to such trypsin levels that are enough to activate PAR-2 receptor on the same cancer cells in possible autocrine/paracrine manner<sup>[31]</sup>. It is worth mentioning that trypsin triggers matrilysin (matrix metalloproteinase-7) activity. So it is an important factor of colorectal cancer progression as immunohistochemical labeling of trypsin correlated with the depth of carcinomatous infiltration, lymphatic and venous involvement, metastasis, recurrence and shorter overall survival of patients<sup>[32]</sup>. In our study the activity of cathepsin B could be insufficient to recruit trypsinogen activation in volume that would significantly affect tumor invasion as we detected lower cathepsin B activities in colorectal cancer compared with healthy volunteers.

The role of cathepsin B is supposed to be diverse. Namely, it can promote neoplastic invasion by lysis of EMC protein nets. Furthermore, by paracrine loop, it could cause death of neighboring cancer cells directly by proteolytic damage of cell membranes and changes in structure superficial cell receptors. Indirectly cathepsin B might act as TNF- $\alpha$ -induced apoptotic second messenger that mediates damage of mitochondria and release cytochrome c from these organelles<sup>[33]</sup>. Thus, the components of cellular producer cathepsin B can be responsible for harmful suicidal injury. It can explain why higher serum levels of cathepsin B were associated with smaller size of tumor.

Cathepsin comes from two kinds of cells: carcinomatous ones and inflammatory cells that constitute immunologic response to neoplasm. According to our present results, we

suspect that along with progression of colorectal cancer, inflammatory cells might become significant producers of cathepsins. We observed lower activity of cathepsins in cases of larger tumors (pT3 and pT4 group) in comparison with smaller ones (pT1 and pT2 group) in measurements before surgery. The cause of it, can be a more escalated immunologic reaction in the onset and early stages of cancer than in advanced and late phases of neoplastic disease. Simultaneously, the outflow of cathepsins into extracellular environment destroys stromal protein structure, which is suspected to facilitate neoplastic spreading, but on the other hand, it can influence the vitality of cancer cells. So cathepsins particularly cathepsin B (probably in lower concentrations that do not induce trypsinogen activity) may disable neoplastic cells in such a way that they cannot forcibly invade surroundings despite promoting invasion by proteolysis of the interstitium. This action of cathepsin B is presumably responsible for relatively higher postoperative levels of serum cathepsin B in case of node negative patients *vs* node positive ones.

Blood serum activity of cathepsin B and D depends on the time of sampling, tumor size and lymph node involvement. Significantly increased activity of cathepsin D could indicate malignant colorectal condition. However, decreased activity of cathepsin B indicates that also different mechanisms besides proteolytic ones, participate in cancer invasion and metastasis. In comparison with analogous higher activity in node negative colorectal cancers, the significant downfall of postoperative cathepsin B activity coexisted with cancerous node involvement. Therefore, in our work, the decrease of this serum protease levels appears as an obvious concomitant of local lymph node metastasis the well-known clinicopathological feature of poor prognosis.

## REFERENCES

- 1 Berchem G, Glondou M, Gleizes M, Brouillet JP, Vignon F, Garcia M, Liaudet-Coopman E. Cathepsin-D affects multiple tumor progression steps *in vivo*: proliferation, angiogenesis and apoptosis. *Oncogene* 2002; **21**: 5951-5955
- 2 Briozzo P, Badet J, Capony F, Pieri I, Montcourrier P, Barritault D, Rochefort H. MCF7 mammary cancer cells respond to bFGF and internalize it following its release from extracellular matrix: a permissive role of cathepsin D. *Exp Cell Res* 1991; **194**: 252-259
- 3 Morikawa W, Yamamoto K, Ishikawa S, Takemoto S, Ono M, Fukushima J, Naito S, Nozaki C, Iwanaga S, Kuwano M. Angiostatin generation by cathepsin D secreted by human prostate carcinoma cells. *J Biol Chem* 2000; **275**: 8912-8920
- 4 Glondou M, Coopman P, Laurent-Matha V, Garcia M, Rochefort H, Liaudet-Coopman E. A mutated cathepsin-D devoid of its catalytic activity stimulates the growth of cancer cells. *Oncogene* 2001; **20**: 6920-6929
- 5 Campo E, Munoz J, Miquel R, Palacin A, Cardesa A, Sloane BF, Emmert-Buck MR. Cathepsin B expression in colorectal carcinomas correlates with tumor progression and shortened patient survival. *Am J Pathol* 1994; **145**: 301-309
- 6 Talieri M, Papadopoulou S, Scorilas A, Xynopoulos D, Arnogiannis N, Plataniotis G, Yotis J, Agnanti N. Cathepsin B and cathepsin D expression in the progression of colorectal adenoma to carcinoma. *Cancer Lett* 2004; **205**: 97-106
- 7 Buck MR, Karustis DG, Day NA, Honn KV, Solane BF. Degradation of extracellular-matrix proteins by human cathepsins B from normal and tumor tissues. *Biochem J* 1992; **282**: 273-277
- 8 Ugarova TP, Ljubimov AV, Deng L, Plow EF. Proteolysis regulates exposure of the IIICS-1 adhesive sequence in plasma

- fibronectin. *Biochemistry* 1996; **35**: 10913-10921
- 9 **Tawatari T**, Kawabata Y, Katunuma M. Crystallization and properties of cathepsin B from rat liver. *Eur J Biochem* 1979; **102**: 279-289
  - 10 **Barrett AJ**. Cathepsin D and other carboxyl proteinases In: Proteinases in mammalian cells and tissues. *North Holland Publishing Company* 1977: 240-243
  - 11 **Lah TT**, Kalman E, Najjar D, Gorodetsky E, Brennan P, Somers R, Daskal I. Cells producing cathepsins D, B, and L in human breast carcinoma and their association with prognosis. *Hum Pathol* 2000; **31**: 149-160
  - 12 **Ioachim EE**, Goussia AC, Machera M, Tsianos EV, Kappas AM, Agnantis NJ. Immunohistochemical evaluation of cathepsin D expression in colorectal tumors: a correlation with extracellular matrix components, p53, pRb, bcl-2, c-erbB-2, EGFR and proliferation indices. *Anticancer Res* 1999; **19**: 2147-2155
  - 13 **Matsuo K**, Kobayashi I, Tsukuba T, Kiyoshima T, Ishibashi Y, Miyoshi A, Yamamoto K, Sakai H. Immunohistochemical localization of cathepsins D and E in human gastric cancer: a possible correlation with local invasive and metastatic activities of carcinoma cells. *Hum Pathol* 1996; **27**: 184-190
  - 14 **Chabowski A**, Sulkowska M, Sulkowski S, Famulski W, Skrzydłewska E, Kisielewski W. Immunohistochemical evaluation of cathepsin D expression in colorectal cancer. *Folia Histochem Cytobiol* 2001; **39**: 153-154
  - 15 **Brouillet JP**, Dufour F, Lemamy G, Garcia M, Schlup N, Grenier J, Mani JC, Rochefort H. Increased cathepsin D level in the serum of patients with metastatic breast carcinoma detected with a specific pro-cathepsin D immunoassay. *Cancer* 1997; **79**: 2132-2136
  - 16 **van der Stappen JW**, Williams AC, Maciewicz RA, Paraskeva C. Activation of cathepsin B, secreted by a colorectal cancer cell line requires low pH and is mediated by cathepsin D. *Int J Cancer* 1996; **67**: 547-554
  - 17 **Nishikawa H**, Ozaki Y, Nakanishi T, Blomgren K, Tada T, Arakawa A, Suzumori K. The role of cathepsin B and cystatin C in the mechanisms of invasion by ovarian cancer. *Gynecol Oncol* 2004; **92**: 881-886
  - 18 **Skrzydłewska E**, Kozusko B, Sulkowska M, Bogdan Z, Kozłowski M, Snarska J, Puchalski Z, Sulkowski S, Skrzydłowski Z. Antioxidant potential in esophageal, stomach and colorectal cancers. *Hepatogastroenterology* 2003; **50**: 126-131
  - 19 **Amiguet JA**, Jimenez J, Monreal JJ, Hernandez MJ, Lopez-Vivanco G, Vidan JR, Conchillo F, Liso P. Serum proteolytic activities and antiproteases in human colorectal carcinoma. *J Physiol Biochem* 1998; **54**: 9-13
  - 20 **Kos J**, Nielsen HJ, Krasovec M, Christensen IJ, Cimerman N, Stephens RW, Brunner N. Prognostic values of cathepsin B and carcinoembryonic antigen in sera of patients with colorectal cancer. *Clin Cancer Res* 1998; **4**: 1511-1516
  - 21 **Strojan P**, Budihna M, Smid L, Vrhovec I, Skrk J. Cathepsin D in tissue and serum of patients with squamous cell carcinoma of the head and neck. *Cancer Lett* 1998; **130**: 49-56
  - 22 **Sis B**, Sagol O, Kupelioglu A, Sokmen S, Terzi C, Fuzun M, Ozer E, Bishop P. Prognostic significance of matrix metalloproteinase-2, cathepsin D, and tenascin-C expression in colorectal carcinoma. *Pathol Res Pract* 2004; **200**: 379-387
  - 23 **Nishimura Y**, Kawabata T, Kato K. Identification of latent procathepsins B and L in microsomal lumen: characterization of enzymatic activation and proteolytic processing *in vitro*. *Arch Biochem Biophys* 1988; **261**: 64-71
  - 24 **Ikeda Y**, Ikata T, Mishiro T, Nakano S, Ikebe M, Yasuoka S. Cathepsins B and L in synovial fluids from patients with rheumatoid arthritis and the effect of cathepsin B on the activation of pro-urokinase. *J Med Invest* 2000; **47**: 61-75
  - 25 **Levicar N**, Kos J, Blejec A, Golouh R, Vrhovec I, Frkovic-Grazio S, Lah TT. Comparison of potential biological markers cathepsin B, cathepsin L, stefin A and stefin B with urokinase and plasminogen activator inhibitor-1 and clinicopathological data of breast carcinoma patients. *Cancer Detect Prev* 2002; **26**: 42-49
  - 26 **Kostoulas G**, Lang A, Nagase H, Baici A. Stimulation of angiogenesis through cathepsin B inactivation of the tissue inhibitors of matrix metalloproteinases. *FEBS Lett* 1999; **455**: 286-290
  - 27 **Hirtenlehner K**, Pec M, Kubista E, Singer CF. Influences of stroma-derived growth factors on the cytokine expression pattern of human breast cancer cell lines. *Arch Gynecol Obstet* 2002; **266**: 108-113
  - 28 **Halangk W**, Lerch MM, Brandt-Nedelev B, Roth W, Ruthenburger M, Reinheckel T, Domschke W, Lippert H, Peters C, Deussing J. Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. *J Clin Invest* 2000; **106**: 773-781
  - 29 **Teich N**, Bodeker H, Keim V. Cathepsin B cleavage of the trypsinogen activation peptide. *BMC Gastroenterol* 2002; **2**: 16
  - 30 **Williams SJ**, Gotley DC, Antalis TM. Human trypsinogen in colorectal cancer. *Int J Cancer* 2001; **93**: 67-73
  - 31 **Ducroc R**, Bontemps C, Marazova K, Devaud H, Darmoul D, Laburthe M. Trypsin is produced by and activates protease-activated receptor-2 in human cancer colon cells: evidence for new autocrine loop. *Life Sci* 2002; **70**: 1359-1367
  - 32 **Yamamoto H**, Iku S, Adachi Y, Imsumran A, Taniguchi H, Nosho K, Min Y, Horiuchi S, Yoshida M, Itoh F, Imai K. Association of trypsin expression with tumor progression and matrilysin expression in human colorectal cancer. *J Pathol* 2003; **199**: 176-184
  - 33 **Foghsgaard L**, Wissing D, Mauch D, Lademann U, Bastholm L, Boes M, Elling F, Leist M, Jaattela M. Cathepsin B acts as a dominant execution protease in tumor cell apoptosis induced by tumor necrosis factor. *J Cell Biol* 2001; **153**: 999-1010