

# Significance and prognostic value of lysosomal enzyme activities measured in surgically operated adenocarcinomas of the gastroesophageal junction and squamous cell carcinomas of the lower third of esophagus

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## Abstract

**AIM:** To establish whether there are fundamental differences in the biochemistries of adenocarcinomas of the gastroesophageal junction (GEJ) and the squamous cell carcinomas of the lower third of the esophagus (LTE).

**METHODS:** Between February 1, 1997 and February 1, 2000, we obtained tissue samples at the moment of resection from 54 patients for biochemical analysis. The full set of data could be comprehensively analyzed in 47 of 54 patients' samples (81%). Of these, 29 were adenocarcinomas of the GEJ Siewert type I ( $n = 8$ ), type II ( $n = 12$ ), type III ( $n = 9$ ), and 18 presented as squamous cell carcinomas of the LTE. We evaluated the mean values of 11-lysosomal enzyme and 1-cytosol protease activities of the tumorous and surrounding mucosae as well as their relative activities, measured as the ratio of activity in tumor and normal tissues from the same patient. These data were further analyzed to establish the correlation with tumor localization, TNM stage (lymph-node involvement), histological type (papillary, signet-ring cell, tubular), state of differentiation (good, moderate, poor), and survival ( $\leq 24$  or  $\geq 24$  mo).

**RESULTS:** In adenocarcinomas, the activity of  $\alpha$ -mannosidase (AMAN), cathepsin B (CB) and dipeptidyl-peptidase I (DPP I) increased significantly as compared to the normal gastric mucosa. In squamous cell carcinomas of the esophagus, we also found a significant difference in the activity of cathepsin L and tripeptidyl-peptidase I in addition to these three. There was a statistical correlation of AMAN, CB, and DPP I activity between the level of differentiation of adenocarcinomas of the GEJ and lymph node involvement,

because tumors with no lymph node metastases histologically confirmed as well-differentiated, showed a significantly lower activity. The differences in CB and DPP I activity correlated well with the differences in survival rates, since the CB and DPP I values of those who died within 24 mo following surgical intervention were significantly higher than of those who survived for 2 years or more.

**CONCLUSION:** Adenocarcinomas of the GEJ form a homogenous group from a tumor-biochemical aspect, and differ from the biochemical characteristics of squamous cell carcinomas of the LTE on many points. When adenocarcinomas of the GEJs are examined at the preoperative phase, the ratio of the performed AMAN, CB, and DPP I enzymatic activity of the tissue sample from the tumor and adjacent intact mucosa within 2 cm of the tumor may have a prognostic value even in the preoperative examination period, and may indicate that ranking of these patients into the neo-adjuvant treatment group should be considered.

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**Key words:** Prognostic value; Lysosomal enzymes; Cardiac adenocarcinomas; Siewert classification; Esophageal squamous cell carcinoma

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## INTRODUCTION

While the majority of carcinomas located in the upper and middle third of the esophagus are squamous cell carcinomas, the incidence of adenocarcinomas in the lower third of the esophagus (LTE) is increasing in the Western world. Although their etiology is not clear, esophageal adenocarcinomas almost always arise in areas of Barrett's metaplasia, usually in the specialized intestinal-type mucosa in the lower esophagus, and predominantly affect elderly white men, often those with a history of heavy smoking. Barrett's metaplasia

is an accepted complication of gastroesophageal reflux disease, but gastric cancer is not linked to reflux disease. Thus, it is of interest to note that the incidence of proximal gastric adenocarcinomas is increasing. At Temple University Hospital, the percentage of cardiac carcinomas within gastric carcinomas has increased from 16% to 25% between 1976-80 and 1991-95<sup>[1]</sup>. Antonioli and Goldman<sup>[2]</sup> reported that the incidence of cardiac carcinomas has increased from 0% to 27% of all gastric carcinomas in Boston. They also showed that the number of signet-ring cell carcinomas is increased with a decrease in the male to female ratio, and an increase in the age of patients.

The classification system described by Siewert<sup>[3]</sup>, which organizes adenocarcinomas of the gastroesophageal junction (GEJ) into tumors of the distal esophagus as type I (Si I), true carcinomas of the cardia as type II (Si II) and subcardial carcinomas as type III (Si III), has allowed for a comparative assessment of the data of the various sites and has facilitated the choice of surgical therapy. This classification brings light into the "Black Box" of GEJ carcinomas. The classification itself can easily be performed by summarizing all available information from contrast radiography, endoscopy, and intraoperative findings.

Following surgical resection, there is a clear survival advantage for patients with early-stage tumors over patients with late-stage tumors, regardless of tumor location. There is, therefore, no difference in the survival rates between any of the three locations<sup>[4]</sup>. Although much effort has been made to obtain a more accurate understanding and to apply more effective therapy for carcinomas in the lower esophagus and proximal stomach, patient survival rates are still not satisfactory.

A number of molecular markers have been analyzed as possible prognostic factors in patients with esophageal cancer including expression of proliferating cell nuclear antigen, epidermoid growth factor receptor, cyclin D1, p53, and p21. However, none of these markers are of clinical value<sup>[5-7]</sup>. Merely aberrant expression of pRB and high serum immunosuppressive acidic protein concentration seems to be useful as a prognostic factor for esophageal squamous cell carcinoma<sup>[8,9]</sup>.

As the etiology of tumors may well be different, it is important to establish whether there are fundamental differences in the biochemistries of adenocarcinomas of the GEJ and the squamous cell carcinomas of the LTE. In order to answer this question, it seems evident that proteolytic enzyme activities in the tumor tissue and the histologically intact mucosa bordering it should be measured and compared, since peptidases are primarily responsible for intracellular catabolism and turnover. The increased level of lysosomal enzymes in tumors, together with their ability to degrade extracellular matrix proteins, has led to the hypothesis that they could be involved in the process of invasion and metastasis<sup>[10-12]</sup>.

## MATERIALS AND METHODS

Between February 1, 1997 and February 1, 2000, we obtained tissue samples at the moment of resection from 54 patients for biochemical analysis. The average age was 57.8 years and the M/F ratio was 35/19 (65%/35%).

The full set of data could be comprehensively analyzed in 47 of 54 patients' samples (81%). Of these, 29 were adenocarcinomas of the GEJ Siewert type I ( $n = 8$ ), type II ( $n = 12$ ), type III ( $n = 9$ ), and 18 presented as squamous cell carcinomas of the LTE. Tissue samples obtained during surgery were further separated into two groups: cancer tissue and normal tissue closely surrounding the cancer tissue, within 2 cm of the tumor border. The latter was checked histologically to confirm it to be tumor-free. For enzyme assays the mucosal layer was used. Reasons for exclusion included histologically confirmed synchronous multiple carcinoma ( $n = 3$ ), death within early postoperative period ( $n = 2$ ) and intolerance of study protocol ( $n = 2$ ).

All patients in the study were so-called advanced cancer cases. Thus, the TNM stage and number of esophageal tumors were IIA:6, IIB:6, and III:6, while the tumors of the GEJ were II:10, IIIA:11, and IIIB:8.

The central-European reality is that almost 90% of malignant carcinomas in this area of the gastrointestinal tract are discovered at an advanced stage, and this fact is well reflected in this group of patients.

We evaluated the mean values of 11-lysosomal enzyme and 1-cytosol protease (as control) specific activities of the tumorous and surrounding mucosae as well as their relative activities, measured as the ratio of activity in tumor and normal tissues from the same patient. These data were further analyzed to establish the correlation with tumor localization, TNM stage (lymph-node involvement), histological type (papillary, signet-ring cell, tubular), state of differentiation (good, moderate, poor), and survival ( $\leq 24$  or  $\geq 24$  mo). The term of relative activity was used for the mean ratio of enzyme activity values for tumorous and intact mucosae in individual patients expressed as a decimal fraction.

Tissue samples from the tumor and intact surrounding area were frozen on dry ice immediately after dissection and stored at  $-70\text{ }^{\circ}\text{C}$  prior to use.

Samples were thawed on ice, placed in 50 volumes (w/v) of 0.15 mol/L NaCl, 0.1% Triton X-100 and homogenized with a Brinkmann Polytron homogenizer. A soluble supernatant was prepared by centrifugation at 12 000  $g$  for 25 min at  $4\text{ }^{\circ}\text{C}$ .

Glycosidase activities were measured using 4-methylumbelliferyl (4-MU) substrates as previously described<sup>[13]</sup>. Protease assays using 7-amino-4-methylcoumarine (AMC) substrates were conducted as described by Sleat *et al.*<sup>[14]</sup>, and Sohar *et al.*<sup>[15]</sup>. Reactions were initiated by adding 40  $\mu\text{L}$  of substrate (various concentrations 20  $\mu\text{mol/L}$ -1 mmol/L)-buffer (100 mmol/L) solution to 5  $\mu\text{L}$  (cathepsin B) or 10  $\mu\text{L}$  (other enzymes) of sample (supernatants diluted two-, four-, and eightfold in homogenization buffer in duplicate), incubated at  $37\text{ }^{\circ}\text{C}$ , and terminated by the addition of 100  $\mu\text{L}$  of 0.5 mol/L glycine, pH 10.5 (4-MU substrates) or 0.1 mol/L monochloroacetic acid in 0.1 mol/L acetate, pH 4.3 (AMC substrates). Buffers consisted of 0.1 mol/L citric acid or 0.1 mol/L sodium acetate adjusted to the indicated pH using sodium hydroxide, acetic acid, or HCl respectively, contained 0.1% Triton-X-100 with 0.15 mol/L NaCl. Substrates were purchased from Sigma and prepared as stocks in dimethyl sulfoxide (remaining substrates) that

were added to the reaction buffer immediately prior to assay. Samples added to substrate solutions after the addition of the termination buffer were used as blanks. Fluorescent reaction products were determined using a CytoFluor II fluorescence multiwell plate reader (PerSeptive Biosystems, Framingham, MA, USA) with excitation at 360 nm and emission at 460 nm.

Since our variables were normally distributed, Microsoft Excel *t*-test with two samples, correlated *t*-test and single factor analysis of variance were used for the statistical analysis. Permission for the investigations was sought and obtained from the appropriate local ethical committee.

**RESULTS**

The analysis of 11-lysosomal enzyme and 1-cytosol protease-thimet oligopeptidase (THP)-activity levels in adenocarcinomas of the GEJ and squamous cell carcinomas of the LTE showed that  $\alpha$ -mannosidase (AMAN), cathepsin B (CB) and dipeptidyl-peptidase I (DPP I) had a significant increase in adenocarcinoma (Table 1). AMAN, CB, DPP I, cathepsin L (CL) and tripeptidyl-peptidase I (TPP I) showed a significant increase in squamous cell carcinoma as compared to the levels of activity measured in the bordering intact mucosa (Table 2).

When relative activity values were compared (Table 3) for  $\alpha$ -glucosidase (AGLU), CB, CL, and DPP I enzymes, the activity level was twofold higher in squamous carcinomas than in adenocarcinomas.

The values of peptidase activity in cardiac tumors with Si I, II, and III localization did not differ significantly (Table 4). There was no significant correlation between lysosomal enzyme activities and adenocarcinomas of different histological types (papillary, signet-ring cell, tubular). There was no significant difference between carcinomas of stages II, IIIA, and IIIB. However, we found a significant and relatively strong relationship between the level of differentiation of adenocarcinomas of the GEJ and the level of activity of AMAN (H: 0.78), CB (H: 0.76), and DPP I (H: 0.67) lysosomal enzymes. The lysosomal enzyme activities in well-differentiated adenocarcinomas were significantly lower than those in poorly differentiated ones. The changes of the activity of certain enzymes correlated well with lymph node involvement. Tumors with no lymph-node metastases showed a significantly lower value of activity. A moderate relation was detected between the relative activities and the presence of lymph-node metastases (H: 0.61). The change in CB and DPP I activity sensitively reflected the differences in survival rates too.

Tables 2 and 3 show that the lysosomal enzyme activity increased significantly in the so-called advanced esophageal carcinomas compared to that in adenocarcinomas of the GEJ. However, we found no statistically measurable correlation between the rate of increased lysosomal enzyme activities and maturity of tumors, as well as the involvement of lymph nodes and TNM stage. Moreover, in contrast to adenocarcinomas of the GEJ, the significantly increased lysosomal enzyme activities in squamous cell carcinomas of the LTE did not correlate with survival time. Thus, these parameters in stages II and III had no prognostic value.

**Table 1** Specific activities of lysosomal enzymes in 29 adenocarcinomas of the GEJ and surrounding normal mucosa (mean $\pm$ SE)

Enzymes	Adenocarcinoma	Normal gastric mucosa	P
AGLU	27 315 $\pm$ 3 139	31 210 $\pm$ 2 490	NS
AMAN	89 594 $\pm$ 8 518	66 653 $\pm$ 4 066	<0.05
BGAL	318 224 $\pm$ 43 694	245 442 $\pm$ 15 421	NS
CB	324 095 $\pm$ 39 402	167 582 $\pm$ 14 842	<0.001
CL	392 438 $\pm$ 44 328	296 166 $\pm$ 29 044	NS
DPPI	2 140 499 $\pm$ 287 604	1 291 759 $\pm$ 126 796	<0.05
CH	491 813 $\pm$ 51 110	404 285 $\pm$ 40 939	NS
DPPII	80 982 $\pm$ 9 916	110 728 $\pm$ 15 265	NS
GCU	219 615 $\pm$ 42 364	178 776 $\pm$ 18 607	NS
HEX	213 292 $\pm$ 28 785	186 396 $\pm$ 18 779	NS
TPPI	921 939 $\pm$ 328 332	443 130 $\pm$ 134 508	NS
THP	36 271 $\pm$ 14 639	23 382 $\pm$ 10 817	NS

GEJ: gastroesophageal junction; NS: non significant; AGLU:  $\alpha$ -glucosidase; AMAN:  $\alpha$ -mannosidase; BGAL:  $\beta$ -galactosidase; CB: cathepsin B; CL: cathepsin L; DPP I: dipeptidyl-peptidase I; CH: cathepsin H; DPP II: dipeptidyl-peptidase II; GCU:  $\beta$ -glucuronidase; HEX:  $\beta$ -hexosaminidase B; TPPI: tripeptidyl-peptidase I; THP: thimet oligopeptidase (pmol /h mg).

**Table 2** Specific activities of lysosomal enzymes in 18 squamous cell carcinomas of the LTE and surrounding normal mucosa (mean $\pm$ SE)

Enzymes	Squamous cell carcinoma	Normal esophageal mucosa	P
AGLU	54 216 $\pm$ 16 793	21 596 $\pm$ 4 106	NS
AMAN	86 987 $\pm$ 14 625	36 905 $\pm$ 2 287	<0.05
BGAL	225 913 $\pm$ 43 791	135 696 $\pm$ 24 644	NS
CB	535 860 $\pm$ 134 809	114 697 $\pm$ 13 037	<0.05
CL	292 996 $\pm$ 56 644	60 964 $\pm$ 14 115	<0.001
DPPI	2 328 558 $\pm$ 634 873	401 436 $\pm$ 172 042	<0.05
CH	578 119 $\pm$ 50 491	453 922 $\pm$ 54 228	NS
DPPII	41 816 $\pm$ 7 226	34 539 $\pm$ 6 833	NS
GCU	134 414 $\pm$ 22 391	115 250 $\pm$ 19 634	NS
HEX	149 704 $\pm$ 30 362	86 965 $\pm$ 15 449	NS
TPPI	191 061 $\pm$ 20 874	112 048 $\pm$ 11 472	<0.001
THP	45 828 $\pm$ 7 921	31 459 $\pm$ 7 164	NS

LTE: lower third of the esophagus; NS: non significant; AGLU:  $\alpha$ -glucosidase; AMAN:  $\alpha$ -mannosidase; BGAL:  $\beta$ -galactosidase; CB: cathepsin B; CL: cathepsin L; DPP I: dipeptidyl-peptidase I; CH: cathepsin H; DPP II: dipeptidyl-peptidase II; GCU:  $\beta$ -glucuronidase; HEX:  $\beta$ -hexosaminidase B; TPPI: tripeptidyl-peptidase I; THP: thimet oligopeptidase (pmol /h mg).

**Table 3** Relative activities of lysosomal enzymes in adenocarcinoma of the GEJ and in squamous cell carcinoma of the LTE (mean $\pm$ SE)

Enzymes	GEJ Tumor/own normal mucosa	LTE Tumor/own normal mucosa	Ratio of LTE/GEJ
AGLU	0.91 $\pm$ 0.09	2.55 $\pm$ 0.78	2.8
AMAN	1.39 $\pm$ 0.12	2.36 $\pm$ 0.32	1.7
BGAL	1.33 $\pm$ 0.16	2.01 $\pm$ 0.42	1.5
CB	2.15 $\pm$ 0.34	4.89 $\pm$ 1.20	2.3
CL	1.48 $\pm$ 0.17	6.21 $\pm$ 2.00	4.2
DPPI	2.34 $\pm$ 0.52	12.43 $\pm$ 5.30	5.3
CH	1.30 $\pm$ 0.12	1.44 $\pm$ 0.31	1.1
DPPII	0.83 $\pm$ 0.08	1.39 $\pm$ 0.23	1.7
GCU	1.36 $\pm$ 0.24	1.46 $\pm$ 0.33	1.1
HEX	1.29 $\pm$ 0.18	2.06 $\pm$ 0.45	1.6
TPPI	1.66 $\pm$ 0.33	1.77 $\pm$ 0.21	1.1
THP	2.36 $\pm$ 0.41	3.59 $\pm$ 2.30	1.5

GEJ: gastroesophageal junction; LTE: lower third of the esophagus; SE: standard error; AGLU:  $\alpha$ -glucosidase; AMAN:  $\alpha$ -mannosidase; BGAL:  $\beta$ -galactosidase; CB: cathepsin B; CL: cathepsin L; DPP I: dipeptidyl-peptidase I; CH: cathepsin H; DPP II: dipeptidyl-peptidase II; GCU:  $\beta$ -glucuronidase; HEX:  $\beta$ -hexosaminidase B; TPPI: tripeptidyl-peptidase I; THP: thimet oligopeptidase.

**Table 4** Prognostic value of lysosomal enzyme activities in adenocarcinomas of the GEJ (mean±SE)

Prognostic categories	Relative activity	AMAN	Specific activity	Relative activity	CB	Specific activity	Relative activity	DPP I	Specific activity
Siewert I	1.36±0.19	NS	110 136±20 902	1.68±0.37	NS	273 838±66 573	1.37±0.42	NS	1 896 366±446 446
Siewert II	1.49±0.23		88 413±17 021	2.42±0.48		417 978±65 610	2.58±0.64		2 673 743±638 594
Siewert III	1.34±0.19		79 101±9 469	2.20±0.68		278 994±63 462	2.68±1.1		1 861 382±384 367
Papillary	1.54±0.27	NS	92 754±22 466	2.79±1.19	NS	368 700±112 886	2.97±1.60	NS	2 156 354±763 729
Signet-ring cell	1.42±0.13		82 743±10 810	2.04±0.27		291 588±58 097	2.37±0.59		2 328 450±567 443
Tubular	1.30±0.26		90 069±8 722	1.80±0.45		325 476±68 549	2.10±1.12		1 916 498±504 487
Grade 1	0.91±0.11	P<0.01	61 931±8 716	0.95±0.08	P<0.01	210 697±54 630	0.72±0.06	P<0.01	981 478±111 507
Grade 2-3	1.63±0.13		103 425±10 283	2.75±0.42		380 793±46 390	3.14±0.69		2 720 009±332 437
N0	1.00±0.17	P<0.05	67 068±10 377	1.03±0.11	P<0.01	201 550±55 664	0.75±0.07	P<0.01	1 053 122±122 928
N1-N2	1.59±0.13		100 857±10 644	2.71±0.43		385 367±44 735	3.12±0.69		2 684 187±345 227
Survival ≤2 yr	1.55±0.14	NS	103 466±11 103	2.76±0.46	P<0.01	382 830±49 621	3.24±0.74	P<0.01	2 722 922±373 145
Survival ≥2 yr	1.13±0.17		67 052±9 135	1.16±0.14		228 650±51 792	0.87±0.07		1 194 060±160 577
Stage II	1.19±0.13	NS	80 194±8 553	1.90±0.50	NS	293 720±52 833	1.98±0.74	NS	1 860 244±358 265
Stage III	1.66±0.18		102 128±15 960	2.47±0.41		364 594±59 832	2.81±0.73		2 514 172±465 458

GEJ: gastroesophageal junction; NS: non significant; N0: lymph node negative; N1-N2: lymph node positive; AMAN:  $\alpha$ -mannosidase; CB: cathepsin B; DPP I: dipeptidyl-peptidase I.

## DISCUSSION

The incidence of adenocarcinomas of the esophagus (Si type I) is increasing<sup>[16,17]</sup>. These cancers are associated with Barrett's specialized epithelium and appear to be part of the continuum of gastroesophageal reflux disease. To date, no data suggest that the prevalence of gastroesophageal reflux disease has changed over the past 20 years and thus the cause of this current "epidemic" remains unclear<sup>[18]</sup>.

The epidemiology of adenocarcinomas of the "classic" gastric cardia (Si type II-III) remains even less clear. Very few studies have examined this as a distinct entity. Overall, it seems that the incidence of cancer in this location, mainly Si type II, has not changed significantly in the past 50 years<sup>[1]</sup>. Some cases of gastric cardia cancer may be associated with chronic gastritis and *Helicobacter pylori* infection, whereas other cases may be related to Barrett's specialized epithelium in the proximal stomach<sup>[19-21]</sup>.

A number of reports suggest that the synthesis, transport, and processing of lysosomal enzymes and their phosphorylated derivatives are altered in cancer, making these proteins interesting subjects for investigation<sup>[22]</sup>. The ability of tumor cells to invade tissues and metastasize is thought to involve an increased expression of proteinases and/or a decrease in the levels of proteinase inhibitors. Peptidases may facilitate metastasis in a number of different ways, including detachment of individual cells from the primary tumor, invasion of surrounding tissues to allow contact with vascular channels, degradation of the basement membrane during both intravasation and extravasation, and invasion of tissues during the formation of secondary tumor sites. Several classes of proteinases have been implicated in this process including matrix metalloproteinases, cysteine proteinases as CB or CL, aspartic proteinase cathepsin D, and serine proteinase plasminogen activators<sup>[23-28]</sup>. It is possible that human tumors may use combinations of these enzymes working synergistically to facilitate invasion or alternatively one specific proteinase may play a dominant role in tissue invasion for a given cancer, while some tumors exhibit an alteration in the synthesis or processing of many or all lysosomal enzymes. Defining the role of proteinases

in the invasiveness of different tumors is important with regard to understanding the biology of this process and searching for potential prognostic markers and targets for therapeutic intervention.

The results of clinical investigations on cysteine cathepsins and their endogenous inhibitors in human breast, lung, brain, liver, and head and neck tumors, as well as in body fluids of ovarian, uterine, melanoma and colorectal carcinoma bearing patients, have shown that these molecules are highly predictive for the length of survival and may be used for risk assessment of relapse and death in cancer patients<sup>[29-32]</sup>.

By examining the correlation of cysteine protease CB and laminin degradation, Khan *et al.*<sup>[33]</sup>, demonstrated that increased CB expression and decreased tumor-associated laminin levels may suggest a mechanism underlying the progression of colorectal adenomas to carcinomas.

As for the relationship of gastric tumors and proteolytic enzymes,  $\beta$  hexosaminidase and its isoenzymes<sup>[34]</sup>, and plasminogen activators are in the center of interest. Cathepsins B, L and tissue type plasminogen activator activities are higher in gastric cancer tissues than in normal gastric tissues. Chung and Kawai<sup>[35]</sup> also revealed that inhibitory activities of CL, CB, urinary and tissue type plasminogen activators are stronger in normal tissue closely surrounding the gastric cancer compared to normal tissue distant from the tumor border, as a result of a defense mechanism of the host against cancer invasion.

Yamamoto *et al.*<sup>[36]</sup>, reported that the matrix metalloproteinase matrilysin may play a key role in the progression of esophageal carcinoma and that its detection may be useful in predicting recurrence and poor prognosis, and possibly in selecting patients for anti-matrix metalloproteinase therapy. Another study on esophageal adenocarcinoma demonstrated that amplicon at 8p22, the locus of the CB gene, is identified and associated with amplification and overexpression of the CB gene in esophageal adenocarcinoma<sup>[37]</sup>.

Prior to this study, no lysosomal enzyme activity measurements have been performed in adenocarcinomas of the GEJ. During our examinations we measured the

activity of 11 different lysosomal enzymes and one cytosol protease THP in Si type I-III adenocarcinomas of the GEJ and in squamous cell carcinomas of the LTE. The activity of AMAN, CB, and DPP I in adenocarcinoma increased significantly as compared to that in normal gastric mucosa. In squamous cell carcinomas of the esophagus (Table 1) we also found a significant difference in the activity of CL and TPP I in addition to these three (Table 2). Moreover, when the relative activity values were compared for AGLU, CB, CL, and DPP I in adenocarcinoma of the GEJ and in squamous cell carcinoma of the LTE, marked differences in the relative activity levels by a factor of 2-5 respectively could be observed in squamous cell carcinomas as opposed to adenocarcinomas (Table 3).

From a tumor-biochemical aspect, adenocarcinomas within the Si I-III localization system did not show any differences, nor were there any significant differences in their lysosomal enzyme activities. Similarly to this there was no significant correlation between protease activities and adenocarcinomas of different histological types (papillary, signet-ring cell, tubular). However, we found a statistically measurable correlation to AMAN, CB and DPP I between the level of differentiation of adenocarcinomas of the GEJ and lymph node involvement, because histologically well-differentiated tumors with no lymph node metastases showed a significantly lower activity. The change of two proteases with cysteine catalytic site, namely CB and DPP I, activity correlated well with differences in survival rates, since the CB and DPP I values of those who died within 24 mo following surgical intervention were significantly higher than the same values for those who survived for 2 years or more (Table 4). At the same time, no statistically measurable correlation was found in relation to the proteases despite of displaying significantly higher activity levels as compared to the normal mucosa, the level of differentiation of tumors, their lymph node state or survival rates for squamous cell carcinomas of the LTE.

We conclude that adenocarcinomas of different locations in the GEJ form a homogenous group and differ in many aspects from malformations and neoformations within similar locations, though they possess a different histological structure. This conclusion is supported by data from Izutani *et al.*<sup>[38]</sup>. The difference in radiation sensitivity could be attributed to the tissue type difference. The increased MnSOD mRNA and MnSOD proteins in adenocarcinoma are believed to indicate the activity of strong defense mechanisms protecting the active form of cysteine catalytic center at CB and DPP I against reactive oxygen species, and this activity could be a cause of resistance against radiation therapy and quinone anticancer drugs.

Our results suggest that when adenocarcinomas of the GEJ are investigated at the preoperative phase, the ratio of the actual AMAN, CB, and DPP I enzyme activity in the samples taken from the tumor and its adjacent tissues may have some prognostic value. Relative activity values within the range of 1, signaling a comparatively more favorable prognosis pattern mean that a specific kind of "static warfare" prevails between the tumor tissue and the surrounding intact mucosa. This may be due not only to the diminished invasiveness of a given tumor, but also to

the more effective defensive properties of the intact bordering mucosa, or it may be a consequence of a reduction in protease inhibitor synthetic activity. It seems, therefore, that the activity levels of AMAN, CB, and DPP I in adenocarcinoma may assist us in formulating our preoperative therapeutic strategy for adenocarcinomas of the GEJ.

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## REFERENCES

- 1 **Locke GR**, Talley NJ, Carpenter HA, Harmsen WS, Zinsmeister AR, Melton LJ. Changes in the site- and histology-specific incidence of gastric cancer during a 50-year period. *Gastroenterology* 1995; **109**: 1750-1756
- 2 **Antonioli DA**, Goldman H. Changes in the location and type of gastric adenocarcinoma. *Cancer* 1982; **50**: 775-781
- 3 **Siewert JR**, Feith M, Werner M, Stein HJ. Adenocarcinoma of the esophagogastric junction. Results of surgical therapy based on anatomical/topographic classification in 1.002 consecutive patients. *Ann Surg* 2000; **232**: 353-361
- 4 **Clark GWB**, Smyrk TC, Burdiles P, Hoefft SF, Peters JH, Kiyabu M. Is Barrett's metaplasia the source of adenocarcinomas of the cardia? *Arch Surg* 1994; **129**: 609-614
- 5 **Hirai T**, Kuwahar M, Yoshida K, Osaki A, Toge T. The prognostic significance of p53, p21 (Waf1, Cip1), and cyclin D1 protein expression in esophageal cancer patients. *Anticancer Res* 1999; **19**: 4587-4591
- 6 **Wang LS**, Chow KC, Chi KH, Liu CC, Li WY, Chiu JH. Prognosis of esophageal squamous cell carcinoma: analysis of clinicopathological and biological factors. *Am J Gastroenterol* 1999; **94**: 1933-1940
- 7 **Zafirellis K**, Dolan K, Fountoulakis A, Dexter SPL, Martin IG, Sue-Ling HM. Multivariate analysis of clinical, operative and pathologic features of esophageal cancer: who needs adjuvant therapy? *Dis Esoph* 2002; **15**: 155-159
- 8 **Shinohara M**, Aoki T, Sato S, Takagi Y, Osaka Y, Koyanagi Y. Cell cycle-regulated factors in esophageal cancer. *Dis Esoph* 2002; **15**: 149-154
- 9 **Shimada H**, Nabeya Y, Okazumi S, Matsubara H, Miyazawa Y, Shihatori T, Hayashi H, Aoki T, Sugaya M, Guniji Y, Kobayashi S, Ochiai T. Prognostic value of preoperative serum immunosuppressive acidic protein in patients with esophageal squamous cell carcinoma. *Dis Esoph* 2003; **16**: 102-106
- 10 **Mullins DE**, Rohrllich ST. The role of proteinases in cellular invasiveness. *Biochim Biophys Acta* 1983; **695**: 177-214
- 11 **Sloane BF**, Hohn KV. Cysteine proteinases and metastasis. *Cancer Metastasis Rev* 1984; **3**: 249-263
- 12 **Duffy MJ**, Ogrady P. Plasminogen activator and cancer. *Eur J Cancer Clin Oncol* 1984; **20**: 577-582
- 13 **Sleat DE**, Sohar I, Lackland H, Majercak J, Lobel P. Rat brain contains high levels of mannose-6-phosphorylated glycoproteins including lysosomal enzymes and palmitoyl-protein thioesterase, an enzyme implicated in infantile neuronal lipofuscinosis. *J Biol Chem* 1996; **271**: 19191-19198
- 14 **Sleat DE**, Sohar I, Pullarkat PS, Lobel P, Pullarkat RK. Specific alterations in levels of mannose 6-phosphorylated glycoproteins in different neuronal ceroid lipofuscinoses. *Biochem J* 1998; **334**: 547-551
- 15 **Sohar I**, Lin L, Lobel P. Enzyme-based diagnosis of late in-

- fantile neuronal ceroid lipofuscinosis: comparison of tripeptidyl peptidase I and pepstatin-insensitive protease assays. *Clin Chem* 2000; **46**: 1005-1008
- 16 **Blot WJ**, Devesa SS, Kneller RW, Fraumeni JF Jr. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. *JAMA* 1991; **265**: 1287-1289
- 17 **Siewert JR**, Feith M, Werner M, Stein HJ. Adenocarcinoma of the esophagogastric junction. Results of surgical therapy based on anatomical/topographic classification in 1,002 consecutive patients. *Ann Surg* 2000; **232**: 353-361
- 18 **Okabayashi T**, Gotoda T, Kondo H, Inui T, Ono H, Saito D. Early carcinoma of the gastric cardia in Japan. Is it different from that in the West? *Cancer* 2000; **89**: 2555-2559
- 19 **Appelman HD**, Kalish RJ, Clancy PE, Orringer MB. Distinguishing features of adenocarcinoma in Barrett's esophagus and in the gastric cardia. In: Speckler S J, Goyal RK, eds. Barrett's esophagus: pathophysiology, diagnosis and management. New York: Elsevier Science Publishing Co Inc 1985
- 20 **Parsonnet J**, Freidman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131
- 21 **Forman D**, Newell DG, Fullerton F, Yarnell JWG, Stacey AR, Wald N. Association between infection with *Helicobacter pylori* and risk of gastric cancer. Evidence from a prospective investigation. *Br Med J* 1991; **302**: 1302-1305
- 22 **Boyer MJ**, Tannock IF. Lysosomes, lysosomal enzymes and cancer. *Adv Cancer Res* 1993; **60**: 269-291
- 23 **Liotta LA**, Stetler-Stevenson WG. Metalloproteinases and cancer invasion. *Semin Ance Biol* 1990; **1**: 99-106
- 24 **Koblinski JE**, Ahram M, Sloane BF. Unraveling the role of proteases in cancer. *Clin Chim Acta* 2000; **291**: 113-135
- 25 **Premzl A**, Puizdar V, Zavasnik-Bergant V, Kopitar-Jerala N, Lah TT, Katunuma N. Invasion of ras-transformed breast epithelial cells depends on the proteolytic activity of cysteine and aspartic proteinases. *Biol Chem* 2001; **382**: 853-857
- 26 **Levicar N**, Nutall RK, Lah TT. Proteases in brain tumour progression. *Acta Neurochir* 2003; **145**: 825-838
- 27 **Yan S**, Sloane BF. Molecular regulation of human cathepsin B: Implication in pathologies. *Biol Chem* 2003; **384**: 845-854
- 28 **Turk V**, Turk B, Guncar G, Turk D, Kos J. Lysosomal cathepsins: structure, role in antigen processing and presentation, and cancer. *Adv Enzyme Reg* 2002; **42**: 285-303
- 29 **Lah TT**, Kos J. Cysteine proteinases in cancer progression and their clinical relevance for prognosis. *Biol Chem* 1998; **379**: 125-130
- 30 **Lah TT**, Cercek M, Blejec A, Kos J, Gorodetsky E, Somers R, Daskal I. Cathepsin B, a prognostic indicator in lymph node-negative breast carcinoma patients: comparison with cathepsin D, cathepsin L, and other clinical indicators. *Clin Cancer Res* 2000; **6**: 578-584
- 31 **Ledakis P**, Tester WT, Rosenberg N, Romero-Fischmann D, Daskal I, Lah TT. Cathepsin D, B, and L in malignant human lung tissue. *Clin Cancer Res* 1996; **2**: 561-568
- 32 **Gardett EA**, Reed MW, Brown NJ. Proteolysis in colorectal cancer. *Mol Pathol* 1999; **52**: 140-145
- 33 **Khan A**, Krishna M, Baker SP, Banner BF. Cathepsin B and tumor associated laminin expression in the progression of colorectal adenoma to carcinoma. *Mol Pathol* 1998; **11**: 704-708
- 34 **Gil-Martin E**, Rodriguez-Berrocal FJ, Paez de la Cadena M, Fernandez-Briera A. N-acetyl-beta hexosaminidase activity and isoenzymes in human gastric adenocarcinoma. *Oncology* 1999; **56**: 142-154
- 35 **Chung SM**, Kawai K. Protease activities in gastric cancer tissues. *Clin Chim Acta* 1990; **189**: 205-210
- 36 **Yamamoto H**, Adachi Y, Itoh F, Iku S, Matsuno K, Kusano M, Arimura Y, Endo T, Hinoda Y, Hosokawa M, Imai K. Association of matrilysin expression with recurrence and poor prognosis in human esophageal squamous cell carcinoma. *Cancer Res* 1999; **59**: 3313-3316
- 37 **Hughes SJ**, Glover TW, Zhu XX, Kuick R, Thoraval D, Orringer MB. A novel amplicon at 8p22-23 results in overexpression of cathepsin B in esophageal adenocarcinoma. *Proc Natl Acad Sci USA* 1998; **95**: 12410-12415
- 38 **Izutani R**, Asano S, Imano M, Kato M, Ohyanagi H, Chihara J. Are adenocarcinomas of the cardia resistant as gastric cancers to quinone anti-cancer drugs and radiation therapy? In: Giuli R, eds. The esophagogastric junction. Paris: John Libbey Eurotext 1998: 1244-1249