Cathepsin B and Cathepsin L Activity Levels in Human Lung and Colorectal Tumor Tissues

İNSAN AKCİĞER VE KOLOREKTAL TÜMÖR DOKULARINDA KATEPSİN B VE KATEPSİN L AKTİVİTE DÜZEYLERİ

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Summary

In this study, activities of cathepsin B (CB) and cathepsin L (CL), the major lysosomal cysteine proteinases, were examined in tumor and corresponding normal tissue samples obtained from patients, either surgically treated or biopsy specimens taken for small cell carcinoma (SCLC), squamous cell carcinoma (SQCLC) of lung, colorectal carcinoma and colorectal adenoma. The correlation between the activity levels of these proteinases with the histological type of the tumor was also investigated. The determination of cathepsin B and L activities in tissue extracts was conducted spectrofotometrically. Tissue CB and CL activity levels were found to be significantly higher in SCLC and SQCLC compared with the matched normal lung parenchyma ($p<0.01$). The comparison of the CB and CL tissue activity levels of SCLC with those of SQCLC showed no statistically significant difference. Colorectal carcinoma tissues have significantly higher CB and CL activity levels compared to both their corresponding non-invaded colorectal mucosa and the colorectal adenoma tissues ($p<0.01$). The data obtained in this study show clearly that; CB and CL are increased in small cell and squamous cell carcinoma of the lung, and colorectal carcinoma, supporting the role of these proteolytic enzymes in malignant progression. However the assay of the activity of these enzymes is not meaningful for the distinction between the two types of bronchial carcinoma.

Key Words: Cathepsins, Small cell lung carcinoma, Squamous cell lung carcinoma, Colorectal carcinoma, Colorectal adenoma

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Özet

Bu çalışmada küçük hücreli ve skuamöz hücreli akciğer karsinomları, kolon karsinomu ve kolon adenomu nedeniyle öpere edilen hastaların tumoral ve normal dokularında başlıca lizozomal sistein proteinazlar olan katepsin B (KB) ve katepsin L (KL) aktiviteleri incelendi. Ayrıca bu proteinazların aktivite düzeyleri ile histopatolojik tümör tipi arasındaki ilişki araştırıldı. Doku özlerindeki KB ve KL aktiviteleri spektrofotometrik yöntemle belirlendi. Küçük hücreli ve skuamöz hücreli akciğer karsinom dokularında KB ve KL aktivite düzeyleri aynı organdan alınan normal dokuya göre istatistiksel olarak anlamlı düzeyde yüksek bulundu ($p<0.01$). Her iki tümör tipi arasında, sözü edilen enzim aktiviteleri açısından anlamlı bir fark bulunamadı. Kolon karsinomlu olgulara ait dokulardaki enzim düzeyleri aynı organdan alınan normal dokulara ve kolon adenomlu olgulardan alınan dokulara göre istatistiksel olarak daha yüksek bulundu ($p<0.01$). Bu çalışmadan elde edilen sonuçlar değerlendirildiğinde, akciğerin küçük hücreli ve skuamöz hücreli karsinomlarında ve kolon karsinomunda saptanan katepsin B ve L düzeylerindeki artışlar, bu proteolitik enzimlerin malignite sürecinde rolü olduğunu ortaya çıkarmaktadır. Ancak bu enzimlerin aktivite düzeyleri her iki tip bronş karsinomunun tanısal ayrımında anlamlı değildir.

Anahtar Kelimeler: Katepsin, Küçük hücreli akciğer karsinomu, Skuamöz hücreli akciğer karsinomu, Kolon karsinomu

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The invasive and metastatic properties of malignant tumors are the principal features underlying the morbidity and mortality of cancer. The metastasizing tumor cell must penetrate the stromal tissue

and the biological barriers, such as basement membranes, in order to enter and leave the circulation. Passage of a cell through such a matrix is considered to involve a three-step process: initial adherence, enzymatic degradation of the matrix components and locomation across the barrier (1,2).

The degradation of matrix components, occurs through the proteolytic enzymes produced and released by tumor cells: mainly; aspartic, serine, and cysteine proteinases. In other words, lysosomal proteinases and metalloproteinases are associated with metastatic processes. Normally, the lysosomal proteinases are safely sequestered behind the lysosomal membrane and participate in a variety of physiological cell functions, e.g. the turnover of intracellular proteins, degradation of internalized proteins by endocytosis, processing of enzyme and hormone precursors. Hence, if they become released either into the cytoplasm or extracellular matrix, they can cause major damage as seen with invasion and metastasis of malignant tumor (3,4).

Cathepsin B and cathepsin L are the major lysosomal cysteine proteinases that have been implicated in local basement membrane dissolution, a prerequisite to tumor invasion. (5). In the last decade, there is an increasing body of literature linking the lysosomal proteinases, especially cathepsins B,L,D,G with lung and colorectal malignancy. Studies on lung and colorectal tumorous tissues as well as tumor cell lines revealed elevated levels of activity or overexpression of these enzymes, particularly cathepsin B and/or cathepsin L. However, most of these studies were performed on a single histological type of tumor (4,6-11). Furthermore, there have been controversial results between the very few investigations evaluating the relationship among the enzymatic activities of both CB and CL, and the tumor histology, in tumorous tissue extracts and their normal counterparts (12-16).

In the present investigation we determined the tissue CB and CL activity levels in four types of primary tumors; small cell lung carcinoma (SCLC), squamous cell lung carcinoma (SQCLC), colorectal carcinoma and colorectal adenoma, and compared the values of each tumor type with the matched adjacent normal tissue values. We aimed to evaluate the metastatic potential of the primary tumor and correlate the activity levels of these proteinases to tumor histology.

Materials and Methods

Patients and tissue samples

Tumor tissue samples were obtained from patients; either surgically treated or biopsy specimens taken for primary tumors; SCLC (n=10), SQCLC (n=10), colorectal carcinoma (n=10) and colorectal adenoma (n=10). All samples were pathologically examined and those tissues surrounding the malignant tissue and diagnosed to be "normal" constituted the normal group. All samples were freed of blood and visible blood vessels, washed in 0.9% NaCl and immediately stored at -70°C before analysis.

This study met the criteria of the local ethical committee.

Preparation of tissue extracts and biochemical analysis of cathepsin B and L

The frozen tissues were minced, thawed on ice and macroscopically homogenous pieces of tissues (O.lg) were taken as specimens. Ten percent tissue homogenates (w/v) prepared in ice cold distilled water using a glass Teflon homogenizer (B.Braun), were frozen and thawed three times for cold extraction. Then the tissue extracts were centrimgated at 17,000 x g for 50 min at +4°C with a Centrikon T 1180.

Cathepsin B and L activities were determined by the spectrophotometric method of Shuja (17). The principle of the method depends on the hydrolysis of the synthetic peptide substrates by CB and CL enzymes in the sample at 37°C. Benzoyl-alanine-arginine-arginine-4-methoxy-2-naphthlyamide (Z-Ala-Arg-Arg-MNA) (pH 6.2) and benzoyl-phenylalanine-arginine-4-methoxy-2-naphthlyamide (Z-Phe-Arg-MNA) (pH 3.5) (Enzymes Systems Products, Dublin CA) were used as synthetic peptide substrates, for CB and CL, respectively. The pink color developed by the reaction of amino acid residues with fast blue B was measured at 520 nm. This color is proportional with the enzyme activity. The enzyme activity is determined as nanomole substrate hydrolyzed per minute. 4-methoxy-2-naphthlyamine was used as standard. The total protein concentration was measured by

Lowry's (18) method using bovine serum albumin as standard. The specific activity results were expressed as nmol/min/mg protein.

Statistical analysis

All values are expressed as the mean plus or minus standard error of mean (SEM). The statistical significance of differences between mean values of cathepsins B and L activities in matched pairs of malignant and normal tissues and mean values of two types of lung carcinoma was calculated by Wilcoxon Matched Pairs Test.

Results

Values of CB and CL activities and statistical significances are shown in Table 1 and Table 2 for the two types of lung carcinoma and colorectal tumors, respectively. Compared with the corresponding non-tumoral lung parenchyma, tissue CB and CL activity levels were found to be significantly higher in small cell carcinoma and squamous cell carcinoma of the lung ($p<0.01$). The mean activities of CB and CL in SCLC tissues were 2.1 fold and 2.0 fold higher than their matched normal lung parenchyma while in SQCLC these differences were 1.64 fold and 1.36 fold. The comparison of the CB and CL activity of SCLC and SQCLC tissues showed no statistically significant differences.

Tissues obtained from patients operated for colorectal carcinoma have significantly higher CB and CL activity levels compared to both their corresponding non invaded colorectal mucosa and the colorectal adenoma tissues ($p<0.01$).

Table 1. Cathepsin B and L activity results (Mean ± SEM) (nmole/min/mg protein) in small cell and squamous cell lung carcinoma tissue samples and normal counterparts

	CB		CL	
Tumor type	N	P	N	P
SCLC (n=10)	25.7 ±9.1	54.2 ± 10.1*	21.6 ±9.0	43.3 ± 3.5*
SQCLC (n=10)	27.6 ± 8.3	45.3 ± 7.9*	16.2 ±9.5	22.0 ± 3.9*

*p<0.01 in comparison to the normal matched tissue (Wilcoxon Matched Pairs Test)
CB=cathepsin B
CL=cathepsin L
N=normal lung parenchyma
P=pathological tissue
SCLC=small cell lung carcinoma
SQCLC= squamous cell lung carcinoma

Table 2. Cathepsin B and L activity results (mean ± SEM) (nmole/min/mg protein) in colorectal adenoma and carcinoma tissue samples and normal counterparts

	CB		CL	
Tumor Type	N	P	N	P
Adenoma (n=10)	27.4 ± 3.4	28.5± 8.2	21.9 ± 3.7	22.0 ± 8.7
Carcinoma (n=10)	34.1 ± 3.6	73.8 ±7.5*	20.4 ± 3.9	32.0 ±7.7*

*p<0.01 in comparison to the normal matched tissue (Wilcoxon Matched Pairs Test)
CB=cathepsin B
CL=cathepsin L
N=normal colorectal mucosa
P=pathological tissue

Discussion

In this study, tissue CB and CL activity levels were found to be significantly higher in small cell and squamous cell carcinoma of the lung compared to the corresponding non-tumoral lung parenchyma. Regarding the histological type of the tumor, although both types of lung carcinomas showed significant differences compared to their normal counterparts, in small cell carcinoma, CB and CL activities were respectively 2.1 fold and 2.0 fold higher in pathological tissues than normal matched tissues; while in squamous cell carcinoma these differences were 1.64 fold for CB and 1.36 fold for CL. Statistically non-significant variation between the two types of bronchial carcinoma might be related to the more invasive and early hematogenous metastatic character of the small cell carcinoma compared to the squamous cell carcinoma of the lung.

In a similiar study of matched pairs of lung tumor tissue and normal lung parenchyma, Ebert et al. (14) have been found CB activities to be increased in tumor tissues and reported insignificantly higher CB activity in adenocarcinoma compared to other histologic cell types. Werle et al. (12) also reported the median activities of CB and CL to be increased in lung tumor tissue compared to non-tumor lung parenchyma. They found insignificantly higher CL activity in adenocarcinoma versus squamous cell carcinoma, while CB activity did not vary across the histologies. They stated that; levels of activity of both enzymes did not correlate with TNM stages nor with cell differentiation of the bronchial carcinomas. Parallel with our results, the CB activity, assayed in tissue extracts, have been found to be significantly higher in squamous cell carcinoma compared to the normal lung parenchyma by Krepela et al (9). According to the results of an immunochemical study performed on samples from squamous cell carcinoma patients, CB have been suggested as an independent prognostic factor for the overall survival of patients suffering from squamous cell carcinoma of the lung (19). In a cell line study; the correlation between CB activity and the in vitro invasive capacity of small cell lung carcinoma cell lines have been shown and the authors conclude that; CB plays an important role in the invasiveness of human small cell lung carcinoma cells (8).

The results of our study and all these studies reveal the role of cathepsins B and L in intracellular and extracellular proteolysis in malignant processes of the lung. Although the determination of their activities in tumor tissues might give idea about the invasive and metastatic potential of the primary tumor and are possibly prognostic markers for the outcome of human lung cancer, Ulbrich et al. (20) stated that, it is not sufficient solely for the diagnosis of histologically different types, possibly because of many other factors involved in malignant processes.

As to our findings related to the colorectal tumors, the tissues belonging to the patients suffering from colorectal carcinoma have significantly higher CB and CL activity levels compared to both their corresponding non invaded colorectal mucosa and the colorectal adenoma tissues obtained from another group of patient.

In a study comparing the cytosolic content of cathepsins B, L and D in a series of matched colorectal carcinoma and adjacent normal colorectal tissues, all studied cathepsins were found to be higher in tumor tissues than in normal mucosa, cathepsin B having the greatest ratio (21). In another study Campo et al (22) observed that cathepsin B expression is up-regulated in human colorectal carcinomas compared with normal mucosa and adenomas. In line with our findings, Shuja et al.(17) observed a significant tumor-specific increase in cathepsin B and L activities with particularly high activity levels in earlier, compared to later stages of colorectal cancer. They also have determined normal levels of cathepsin B in adenomas from colorectal cancer patients. They have suggested that increase in expression of cathepsin B may be a sensitive marker for progression from the pre-malignant to the malignant state in the development of colorectal cancer.

In a study performed on cell lines, the authors observed that the presence of mature cathepsin B coincided with a reduction in pH and an increase in the amount of cathepsin D secreted. As they have mentioned; the simultaneous presence of both a tumor-generated acidic extracellular environment and an elevated secretion of procathepsin D, could result in the activation of latent procathepsin D out-

side the cell (23). Therefore it has been stated that; elevation in cathepsin B activity in colorectal carcinoma has not only a direct role in invasion processes; but it is also effective by causing the activation of latent procathepsin D leading to a further proteolytic hazard in the extracellular matrix components (24).

Our results confirm the above studies and it can be concluded that; CB and CL activity assays in colorectal carcinoma and adenoma tissues is meaningful for the differentiation of malignant versus non-malignant colorectal tumors.

In our study, for all tumor types, CL activity differences between the tumoral and normal tissues are lower than those belonging to CB. These results might be because of the larger activity pH range of CB (3.5-6.0) than CL (5.0-6.1) which will cause a higher CB activity in tumor tissue having mostly acidic pH related to the altered metabolism (23, 25)

The overall conclusion that can be drawn from our study is; the major cysteine proteinases cathepsins B and L are involved in lung and colorectal malignant processes. Although activity levels of cathepsins B and L differ significantly between the carcinoma and adenoma of colorectal tissue, their variance across the small cell and squamous cell carcinoma of the lung is not applicable as an indépendant differentiating criteria for these histologic types.

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