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Increased serum level of membrane type 1-matrix metalloproteinase (MT1-MMP/MMP-14) in patients with breast cancer

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Abstract: Different types of matrix metalloproteinases, including membrane type 1 matrix metalloproteinase (MT1-MMP/MMP-14) can be easily detected in biological fluids and therefore may be contemplated as putative tumor markers. Although increased activity of MT1-MMP/MMP-14 have already been found in breast cancer, little is known about its circulating levels. The aim of the present study was therefore to evaluate serum levels of active form of membrane type 1 matrix metalloproteinase (MT1-MMP/MMP-14). A novel type of activity enzyme-linked immunosorbent assay was used to detect serum levels of MT1-MMP/MMP-14 in 18 patients with invasive ductal breast cancer and 11 healthy controls. In the breast cancer group of patients MT1-MMP/MMP-14 mean (±SD) concentration was 16.91±5.87 ng/ml which was significantly higher ($p<0.0001$) than the mean values obtained for the control i.e. 8.55±1.66 ng/ml. Conclusions: Higher levels of soluble form of MT1-MMP/MMP-14 could play a role in invasiveness and metastasis of breast cancer. Whether or not it has a potential as biochemical marker remains to be determined.

Key words: breast cancer, matrix metalloproteinases, MT1-MMP/MMP-14, serum, ELISA

Introduction

Tumor progression to the malignant phenotype is greatly dependent on the permissive action of the microenviroment. From angiogenesis to inflammation to metastasis, the interactions of cells with their microenviroment provide many of the essential factors. One critical factor regulated by the tumor microenviroment is the production of specific proteolytic enzymes and protease inhibitors capable of altering the immediate pericellular milieu [3]. Membrane type matrix metalloproteinases (MT-MMPs) play a central role in the locomotion of many cell types. MT1-MMP is also known as MMP-14 and is the most common and thoroughly studied member of the MT-MMP subfamily. It is widely expressed in tumors and is frequently associated with enhanced tumorigenicity of many cancer types [4].

At present, little is known about how MT1-MMP/MMP-14 expression is regulated on the plasma membrane. The shedding of membrane-tethered MT1-MMP/MMP-14 from the cell surface, however, offers a concrete mechanism how membrane-bound MMP activity at the cell surface could be regulated. MT-MMPs have a unique regulatory mechanism in which an active enzyme undergoes a series of processing steps, either autocatalytic or mediated by other proteases, that regulate the activity and nature of the enzyme species at the cell surface and at the pericellular space [2]. The processing of active MT1-MMP (57 kDa) is mostly an autocatalytic intermolecular event that results in the generation of an inactive membrane-tethered form of 44 kDa and in the shedding of the catalytic domain.

However, data on circulating MT1-MMP/MMP-14 in patients with breast cancer are lacking. Therefore we attempted to determine soluble MT1-MMP/MMP-14

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Table. 1. Clinicopathological characteristics of breast cancer patients.

No	Age	G	T	N
1.	62	2	2	0
2.	67	3	2	2
3.	55	2	1B	0
4.	71	3	2	2
5.	69	3	2	2
6.	39	2	2	2
7.	54	3	2	1
8.	76	3	2	0
9.	46	3	2	1
10.	61	3	2	0
11.	40	3	2	1
12.	39	3	2	0
13.	31	2	1B	0
14.	49	3	2	0
15.	52	3	1C	1
16.	57	2	1B	0
17.	71	3	2	1
18.	34	2	1B	0

levels in sera of women without clinically apparent metastases.

Materials and methods

Patients. A total of 18 patients (of which four T1b, one T1c and thirteen T2) diagnosed with ductal breast carcinoma but without clinically apparent metastases were involved in this study. 11 healthy controls served as controls. The mean (±SD) age of patients in the study and control groups was similar (54.06±13.02 and 53.56±14.95 years old, respectively).

Serum samples. Sera were obtained prior to surgery according to the ethical standards, with informed consent obtained from every patient. Patients had not received any preoperative chemo- or hormonal therapy. Clinicopathological characteristics of patients are shown in Table 1.

For all patients, the histological diagnosis and the stage of cancer were established by assessment of paraffin sections. Histological grading was performed according to Bloom and Richardson [1]. MT1-MMP/MMP-14 levels were measured by the activity enzyme-linked immunosrobent assay (Amersham Pharmacia Biotech). It uses the preform of a detection enzyme, which is activated by the respective MMP and represents the concentrations of the soluble/active form of the enzyme. The assays' range of detection is 1-32 ng/ml. All measurements were made in duplicate and averaged.

Ethical issues. The study was approved by the Ethics Committee of the Medical University of Bialystok.

Statistical analysis. Student t-test accepting p value less than 0.05 as significant was used for the statistical analysis and assumptions were checked by Kolmogorov test. The analysis was performed by the use of SAS STAT package and Graph Prism.

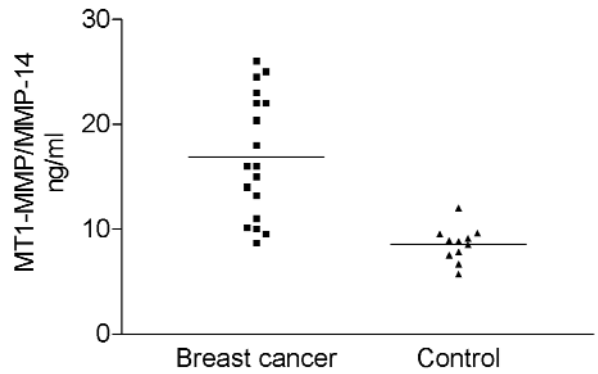

Fig. 1 MT1-MMP/MMP-14 serum concentrations in ductal breast cancer patients and controls.

Results

We found that the mean (±SD) MT1-MMP/MMP-14 concentrations in serum of patients with ductal breast cancer was 16.91±5.87 ng/ml, in comparison with 8.55±1.66 ng/ml in the control group ($p<0.0001$), a statistically significant difference (Fig. 1). No differences in MT1-MMP/MMP-14 levels were found between patients with (N1, N2) and without lymph node involvement, between tumor grade (2, 3) or size (1, 2).

Discussion

Invasion and metastasis require the disruption of several collagen-endowed tissue barriers, key among which is the basement membrane that lines vascular endothelial cells and constitutes a continuous physical obstacle to tumor metastasis. MMPs have long been associated with malignancy [5]. The concentrations of MT1-MMP/MMP-14 in serum represent the levels of active i.e. "shedded" protein. The shedding, by means of overactivity of some types of proteolytic enzymes, including plasmin and other MMPs, may also include release of the entire extracellular extension of MT1-MMP/MMP-14 comprising both the catalytic domain and the C-terminal domain also known as the hemopexin-like domain (HLD). While this process would terminate activity on the plasma membrane independently of exogenous inhibitors, the shed catalytic domain may contribute to pericellular proteolysis.

It seems likely that increased serum concentrations of MT1-MMP/MMP-14 in breast cancer patients may result from accentuated shedding. A feasible explanation is that pro-MT1-MMP/MMP-14 is transported from intracellular compartments to the plasma membrane, then becomes activated by plasmin extracellularly to exert its proteolytic activity. Since overall activity of plasmin in breast cancer patients is increased it is possible that plasmin formation in the tumor might be a contributory factor [6].

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It is difficult to speculate whether activity of the enzyme proceeds or follows the metastasis formation since in our study group half (n=9) of the patients had histologically confirmed lymph node involvement. It is plausible that increased activity of MT1-MMP/MMP-14 might be involved in the enhanced invasiveness at early steps and contribute to subsequent progression of the disease.

In summary, the increased levels of soluble MT1-1/MMP-14 in the serum of breast cancer patients may have implications in the pathogenesis of the disease. Further studies are, however, required to delineate the precise role of soluble MT1-1/MMP-14 in the biology of breast cancer.

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