

## Cathepsins and cystatins in extracellular fluids – useful biological markers in cancer

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*Proteases of all four classes have been shown to participate in processes of tumor growth, vascularisation, invasion and metastasis. Their levels in tumor tissue extracts can provide useful clinical information to predict disease free and overall survival in various types of cancer. Recently we found that cysteine proteinases cathepsins B and H and their endogenous protein inhibitors stefins A, B and cystatin C can also predict prognosis when measured extracellularly. In melanoma and colorectal cancer patients high serum levels of cathepsins as well as high levels of stefins A and B and cystatin C correlated with shorter survival. On the other hand, cathepsin B/cystatin C complex was found to be less abundant in sera of patients with malignant tumors than in those with benign diseases or in healthy controls.*

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### Introduction

Alterations in expression, processing and localization of cysteine proteinases (CPs) in tumor tissues have been observed at various levels when compared to their normal and benign tissue counterparts.<sup>1</sup> CPs can be translocated to the plasma membrane or secreted from tumor cells where they presumably participate in the degradation of components of the extracellular matrix and basement membrane.<sup>1,2</sup> The mechanism of secretion is not fully understood<sup>3</sup>, however, it is known that cathepsins can be secreted from normal and tumor cells as precursors or active enzymes. CPs may be involved also in the formation of new blood vessels, which enable feeding of the growing tumor.<sup>4,5</sup> Relation of mRNA, activity or protein levels of CPs in tumors with clinical characteristics of cancer patients has shown that these molecules are highly predictive for the length of survival and may be

used for assessment of risk of relapse or death for cancer patients.<sup>4</sup> Detection of these proteins in extracellular fluids may extend their application to primary diagnosis<sup>6-8</sup>, to the assessment of response to selected chemotherapy<sup>9</sup> and to the monitoring of malignant disease.

### Quantization of cathepsins and their inhibitors in extracellular fluids

Cathepsins B, H and L have been determined in extracellular fluids of cancer patients by measuring enzymatic activity or immunochemically by ELISA. In first case various chromogenic and fluorogenic substrates and synthetic inhibitors have been used in experimental procedures, resulting in more or less specific signal for individual enzyme. In ELISAs specific monoclonal and polyclonal antibodies, raised to individual human antigens

have been used, providing reliable information of the protein level of each enzyme in extracellular fluids.<sup>10,11</sup> However, for most of cathepsins only the total protein level can be assessed by ELISA, excluding the information on active, pro or complexed enzyme forms. Only for detection of cathepsinB/cystatin C complex a specific ELISA has been designed.<sup>11</sup>

Extracellular levels of the inhibitors of CP have been defined by measuring total cysteine proteinase inhibitor (CPI) activity or by specific ELISAs.<sup>13</sup> Automated particle-enhanced immunoturbidimetric or immunonephelometric assays have been designed for detecting of cystatin C in blood.<sup>12,13</sup>

### **Extracellular cathepsins and their inhibitors as diagnostic or prognostic indicators**

The activity and protein levels of cathepsins and inhibitors have been determined in fluids surrounding tumors, such as bronchoalveolar lavage fluid of lung cancer patients and ascites fluid of ovarian carcinoma patients and in blood and urine.<sup>11</sup>

High levels of cathepsin B have been reported in sera of patients with breast, ovarian, uterine, liver, pancreatic, melanoma, colorectal and lung cancer.<sup>4,11</sup> In patients with colorectal and uterine carcinoma cathepsin B protein or activity levels correlated with tumor stage.<sup>8,14,15</sup> Additionally, in this type of malignancy cathepsin B protein concentration was found to correlate with different modes of patient treatment.<sup>16</sup> Increased levels of cathepsin B were found in urine of patients with gastric cancer<sup>17</sup> but not in urine from breast 18 or bladder carcinoma.<sup>19</sup> Cathepsin B was found as a significant prognostic marker in sera of patients with melanoma<sup>9</sup> and colorectal cancer.<sup>14</sup> Patients with high levels of serum cathepsin B experienced high risk of death.

Cathepsin H was increased in sera of patients with melanoma, colorectal, lung and he-

ad and neck cancer.<sup>11</sup> In melanoma, its protein level was significantly increased within the group of patients who did not respond to the combined chemioimmunotherapy, compared with the group of responders, indicating the potential of this enzyme in predicting the effectiveness of the therapy.<sup>9</sup> In patients with head and neck cancer cathepsin H serum levels correlated with histological grade<sup>20</sup> and in melanoma and lung cancer its high levels correlated with shorter overall survival.<sup>9,21</sup>

Cathepsin L activity levels were found increased in sera of breast, pancreatic, liver and colorectal cancer.<sup>11</sup> Its protein level was found to be increased in sera of patients with ovarian carcinoma and suggested in combination with CA 125 and CA 72-4 as better marker for detection of ovarian cancer than the methods currently used in clinical practice.<sup>7</sup>

Stefin A and stefin B have been detected in ascitic fluid from ovarian carcinoma<sup>22</sup> and in bronchoalveolar fluid of lung cancer patients.<sup>23</sup> Increased serum levels of stefin A in patients with hepatocellular carcinoma and liver cirrhosis correlated with tumor size and with the number of neoplastic lesions.<sup>6</sup> Stefin A, but not stefin B levels were moderately increased also in sera of patients with colorectal and lung cancer.<sup>24</sup> Cystatin C was also increased in sera of cancer patients.<sup>11</sup> In melanoma, colorectal and lung cancer its levels correlated with the progression of the malignant disease. Since cystatin C has also been proposed as an accurate marker of glomerular filtration rate (GFR), its levels in cancer patients should be very carefully evaluated before clinical application of this new GFR marker. Stefin A, stefin B and cystatin C have been reported as significant prognostic markers in sera of patients with colorectal cancer.<sup>24</sup> High levels of all three inhibitors correlated with shorter overall survival although for stefin A the difference between high and low risk patients was not statistically significant. Stefin B was the strongest prognosticator of all three inhibitors and the combi-

nation with cathepsin B or CEA further stratified the risk of death.

In sera of patients with colorectal and lung cancer the level of cathepsin B/cystatin C complex was also determined.<sup>11</sup> The complex was significantly less abundant in sera of patients bearing malignant lung tumors than in those with benign lung diseases or in healthy controls. Similarly, in colorectal cancer sera, its level was lower in Dukes' stages C and D than in early stages A and B. The inverse correlation found in this study between malignant progression and stability of the complex, supports the hypothesis of hindered inhibitory capability during cancer progression.

### Conclusions

Quantization of cysteine cathepsins and their inhibitors in extracellular fluids as compared with tumor tissue cytosols has many advantages. Besides prognostic information their levels can be used also for primary diagnosis, for the assessment of response to selected chemotherapy and for the monitoring of malignant disease. Additionally, the need for careful histological examination of tumor tissue, inherent problems with tissue heterogeneity and problems with the choice of extraction buffer do not apply to extracellular samples. On the other hand, the levels of cathepsins and their inhibitors in blood and other bodily fluids are much lower than in tissue extracts and their assessment requires more sensitive assays. Future activities should be focused on standardization and quality assurance of assays and on definition of subpopulations of cancer patients who would benefit most from the information provided by these new extracellular biological markers.

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