Expression of mRNA MMP-7 and mRNA TIMP-1 in Non-small Cell Lung Cancer

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Abstract. *Background: Destruction of the extracellular matrix is a necessary precondition for metastasis and invasion of tumour cells. Metalloproteinases (MMPs) are involved in this process, matrilysin being one of them (MMP-7). The results of our pilot study with patients operated on for non-small cell lung carcinoma (NSCLC), with the assessment of MMP-7 and the tissue inhibitor of matrix metalloproteinase (TIMP-1), are presented here. Patients and Methods: The group consisted of 34 patients who had been operated on in the course of 2005. Messenger RNA MMP-7 and TIMP-1 were assessed in 20 cases (58%). Tissue samples were frozen to –70˚C, total RNA was subsequently isolated and a reverse transcription was performed from it. The quantitative assessment itself was performed using a real-time PCR method. The resulting expression level was determined as the expression ratio of the assessed gene and the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Results: A higher expression of mRNA MMP-7 was found in the NSCLC tissue than in non-tumourous lung tissue. On the other hand, a higher expression of mRNA TIMP-1 in the non-tumourous surrounding lung tissue was demonstrated. The expression of mRNA MMP-7 and TIMP-1 was higher in adenocarcinoma than in the epidermoid form of NSCLC. Conclusion: The value of our results should not be overestimated since we had only a small group of patients and assessed only one of the whole range of metalloproteinases (MMP-7). We consider the assessment and ratio quantification of metallorpoteinases in normal lung and NSCLC to be the first step in a further application of these parameters.*

Despite the high social seriousness of non-small cell lung carcinoma (NSCLC), the current possibilities for monitoring this disease with tumour markers seem to be insufficient: even CYFRA 21-1 – the best marker for NSCLC – does not achieve satisfactory sensitivity. Tumour markers can be used in particular for the early detection of a relapse of the disease during follow-up, and, with limitations, for therapy control (1, 2). New markers are therefore being sought, which would facilitate the prognosis of NSCLC. At present one of the directions for research and clinical monitoring of tumour markers in general is the assessment of metalloproteinases and their inhibitors (3, 4). The basic manifestation of NSCLC is the ability for invasive growth and metastasis (13). In this process matrix metalloproteinases (MMPs) and their inhibitors (TIPMs) are extensively involved. MMPs are endopeptidases that have the ability to degrade most components of the cellular basal membrane. In the metastatic cascade, these are the factors facilitating tumour cell proliferation. Because of their proteolytic effect, they destroy the extracellular matrix and basal membrane and thus assist the penetration of tumour cells into the surrounding area. They also play a significant role in the process of angiogenesis by creating space for newly arising vessels and by supporting endothelia mobility and invasion of tumour cells into the vessels. Tissue inhibitor of matrix metalloproteinase (TIMP) acts as an antagonist to the proteolytic effect of matrix metalloproteinases. Its effect lies in the stabilization of the basal membrane, which becomes immune to the effect of proteolytic enzymes under the influence of TIMP, and so it makes the invasion of tumour cells and endothelia more difficult (3).

We present the results of our pilot study, with the objective of assessing MMP-7 metalloproteinase expression (matrilysin), as well as the expression of TIMP-1, which is a protein regulating (among other things) the activity of MMP-7. These enzymes were assessed in tissue samples of NSCLC, obtained during surgery.

Patients and Methods

The monitored group consisted of 34 patients, operated on for NSCLC at the Surgical Clinic of the Faculty Hospital in Pilsen, Czech Republic, in 2005. The patients were in Ia-IIIa stages of this

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Table I. *The relative expression of mRNA MMP-7 and TIMP-1 in tumour and non-tumour lung tissue, together for squamous cell carcinoma and adenocarcinoma (median relative values).*

mRNA	Non-tumour tissue (n=20)	Tumour tissue (n=20)
MMP-7/GAPDH	0.0000	0.0040
TIMP-1/GAPDH	0.0127	0.0018

tumour disease, with an average age of 53-74 years (median 65.8 years). All patients had a tumour tissue sample taken during surgery, as well as a sample of tissue not affected by the tumour. All the samples were frozen at a temperature of –70˚C and subsequently overall RNA was isolated with a FAST prep kit (QBiogene, Solon, US). From 3 μg of isolated RNA a reverse transcription was performed. The quantitative assessment itself of mRNA MMP-7 and TIMP-1 expressions was performed with the real-time PCR method from 1 μl cDNA. The resulting level of expression is given as a ratio of the assessed gene to the housekeeping gene, that is a gene expressed in every tissue. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for this purpose.

Results

RNA MMP-7 and TIMP-1 messengers were only detected and consequently assessed in 20 cases (58% of the samples of tumour tissue). Histologically there were 15 tissue samples of squamous cell carcinoma and 5 adenocarcinomas. The results were quantified and subsequently processed with the S.A.S. 8.02 statistical program, US. The results are given in Table I, presenting the expressions of mRNA MMP-7 and TIMP-1 in tumour and non-tumour lung tissue, together for squamous cell carcinoma and adenocarcinoma. In view of the small number of samples in the given group, no statistical significance was achieved in the difference between tumour and non-tumour tissue. Still, on average we demonstrated a higher expression of mRNA MMP-7 in NSCLC tumour tissue than in non-tumourous lung tissue. We also demonstrated that the expression of mRNA TIMP-1 was higher in the surrounding non-tumourous lung tissue than in the tumour itself. Figures 1 and 2 present the expressions of mRNA MMP-7 and TIMP-1 in squamous cell carcinoma and NSCLC adenocarcinoma in the form of box plots. Both figures show a higher expression of mRNA MMP-7 and TIMP-1 in the tissue of squamous cell carcinoma and this was dependent on the stage of the tumour disease (stages I-III). We did not register any major difference between individual stages in the expression of mRNA MMP-7 or TIMP-1 (see Figures 3 and 4).

Discussion

Our results are in accordance with research which also demonstrated a correlation between the aggressiveness of

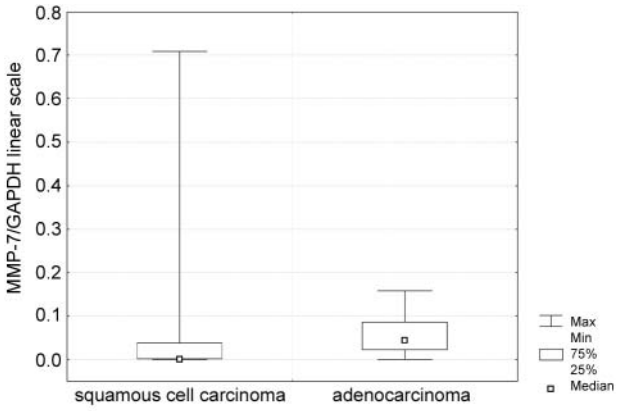

Figure 1. *The box plot of the relative expression of mRNA MMP-7 in the tissue of NSCLC squamous cell carcinoma and adenocarcinoma.*

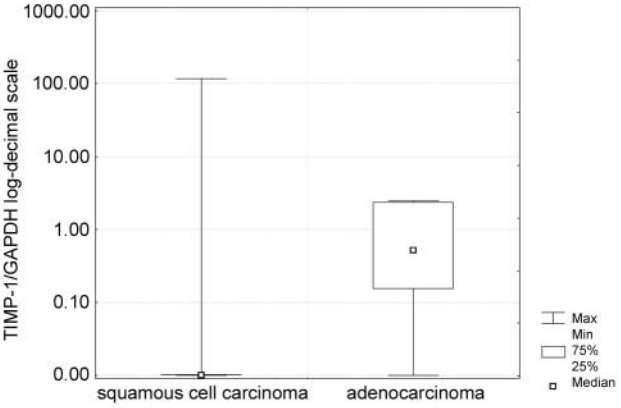

Figure 2. *Box plots of the relative expressions of mRNA TIMP-1 in the tissue of NSCLC squamous cell carcinoma and adenocarcinoma.*

lung tumours and the expression of MMP-7 using immunohistochemical assessment (5-7). According to the present state of knowledge from published papers MMPs and their inhibitors (TIMPs) play an important role in the process of tumour growth and tumour invasiveness in general, including lung cancer or NSCLC (3, 4, 8-11). Through their proteolytic effect they destroy the basal membrane of the extracellular matrix, and thus they assist the invasion and penetration of tumour cells into the healthy tissue. Through this proteolytic effect, tissue proteases also affect the basal membrane of the endothelia, and thus facilitate angiogenesis (new formation of vessels), which is a necessary factor for the expansion and invasion of the primary tumour focus. The activity of metalloproteinases depends on the balance between the level of the active enzymes and their inhibitors (3). Our results mainly confirm the presumed mechanism of metalloproteinases in NSCLC. They also

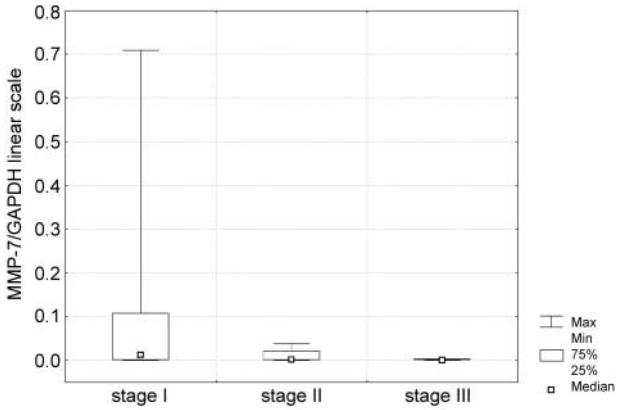

Figure 3. *Box plots of the relativa expression of mRNA MMP-7 in the tissue of NSCLC squamous cell carcinoma and adenocarcinoma depending on the stage of the tumour (stage I-III).*

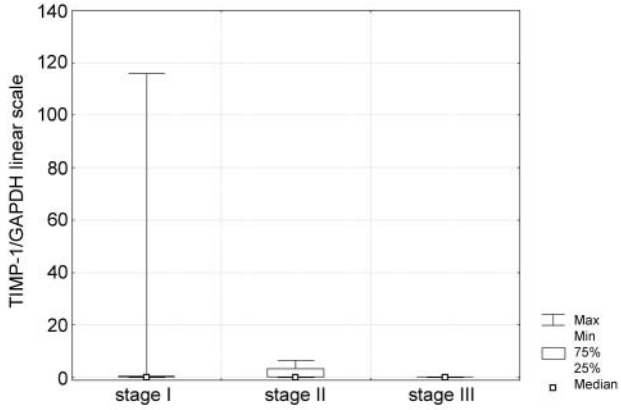

Figure 4. *Box plots of the relative expression of mRNA TIMP-1 in the tissue of NSCLC squamous cell carcinoma and adenocarcinoma depending on the stage of the tumour (stage I-III).*

demonstrated a higher enzymatic activity of metalloproteinases (in our case MMP-7, which is released directly by tumour cells themselves) in tumour tissue, while a higher expression of metalloproteinase inhibitor (TIMP-1) was demonstrated in the adjacent non-tumour tissue, of the resected lung lobe. The small numbers of patients in our group do not enable us to evaluate the demonstrated higher expression of metalloproteinases and their inhibitors in individual histological types (adenocarcinoma, squamous cell carcinoma). In a future study we would like to compare the results of MMP-7 and TIMP-1 tissue expressions with serum levels (12). We believe that an approach comparing expressions between tumourous and non-tumourous tissues of individual patients gives a better representation of the process dynamics than a comparison between patients.

Mainly because of the small group of patients and the assessment of only one out of the whole range of metalloproteinases (MMP-7), the value of our results should not be overstated. We consider the assessment and the ratio quantification of metalloproteinases in lung and tumourous NSCLC tissue to be the first step in a further application of these parameters. Our next effort will be mainly to determine the applicability of these parameters in estimating the invasiveness of tumours or in assessing the prognostic index for individual patients. Unfortunately, the main disadvantage in the practical application of these parameters is still the technical difficulty in their assessment and therefore the very high cost of processing.

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