Expression of Matrix Metalloproteinases 3, 10 and 11 (Stromelysins 1, 2 and 3) and Matrix Metalloproteinase 7 (Matrilysin) by Cancer Cells in Non-Small Cell Lung Neoplasms. Clinicopathologic Studies

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Summary

Matrix metalloproteinases (MMP's) 3, 10 and 11 (also known as stromelysins 1, 2 and 3, respectively), and matrix metalloproteinase 7 (also known as matrilysin), produced by stromal fibroblast-like cells in the vicinity of various malignancies, are suspected to have an ability to degrade components of extracellular matrix, thus promoting spread of the tumor. MMP's also have been found in epithelial tumor cells in various cancers. Tissue sections from 95 cases of non-small cell lung cancer (NSCLC) were immunostained with antibodies against MMP 3, MMP 10 and MMP 11 and sections from 99 cases of NSCLC were immunostained with an antibody against MMP 7. Cytoplasmic immunoreactivity in the tumor cells was semiquantitatively scored for intensity and distribution and correlated with tumor type, tumor grade, stage, tumor size, lymph node positivity, metastasis and survival. Overexpression of MMP 10 and MMP 11 correlated with higher grade for NSCLC (p=0.029 and p=0.016, respectively), and also in a subset of adenocarcinomas (AC) (p=0.015 and p=0.009, respectively). Also, MMP 10 and MMP 11 correlated with lymph node involvement in NSCLC (p=0.025 and p=0.027 respectively). No correlation was found for MMP 3. Overexpression of MMP-7 correlated with tumor stage (p = 0.0001) and was associated with adverse clinical outcome (p = 0.0001) in NSCLC and also in separate squamous cell carcinoma (SCC) (p = 0.003) and AC (p = 0.004) tumor groups.
Key words: **matrix metalloproteinases 3, 10, 11, 7 – non-small cell lung cancer – survival**

Souhrn

Exprese matrixových metaloproteináz 3, 10 a 11 (stromelyzinů 1, 2 a 3) a matrixové metaloproteinázy 7 (matrilyzinu) nádorovými buňkami u nemalobuněčných plicních karcinomů. Klinickopatologické studie

Matrixové metaloproteinázy (MMP) 3, 10 a 11 (rovněž známé jako stromelyziny 1, 2 a 3) a matrixová metaloproteináza 7 (rovněž známá jako matrilyzin) jsou produkovány stromálními buňkami typu fibroblastů v okolí různých maligních tumorů, a předpokládá se, že mají schopnost degradovat komponenty extracelulární matrix, a tak podporovat šíření tumorů. Matrixové metaloproteinázy byly rovněž nalezeny v buňkách různých karcinomů. Tkáňové řezy z 99 případů nemalobuněčných plicních karcinomů byly označeny protilátkami proti MMP 3, 10 a 11 a řezy z 95 případů byly označeny protilátkou proti MMP 7. Intenzita a distribuce cytoplazmické imunoreaktivity byla zhodnocena a statisticky korelována s typem tumoru, stadiem nádoru, gradingem, velikostí tumoru, stavem lymfatických uzlin, metastázami a přežitím. Zvýšená exprese MMP 10 a MMP 11 korelovala s vyšším gradingem v celkové skupině nemalobuněčných tumorů (p = 0,029 a p = 0,016), a také v podskupině adenokarcinomů (p = 0,015 a p=0,009). Rovněž MMP 10 a MMP 11 korelovaly s postižením lymfatických uzlin v celkové skupině nemalobuněčných tumorů (p=0,025 a p = 0,027). Žádná korelace nebyla nalezena pro MMP 3. Zvýšená exprese MMP 7 korelovala se stadiem nádoru (p = 0,0001) a s klinickou progresí v celkové skupině nemalobuněčných tumorů (p = 0,0001) a také v podskupině dlaždicobuněčných karcinomů (p = 0,003) i v podskupině adenokarcinomů (p = 0,004).
Klíčová slova: **matrixové metaloproteinázy 3, 10, 11, 7 – nemalobuněčný karcinom plic – přežití**

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Preliminary results from this work were presented as posters at 91st meeting of The United States and Canadian Academy of Pathology and published in a form of abstracts in *Modern Pathology*.

Matrix metalloproteinases (MMP's) 3, 10 and 11 (also known as stromelysins 1, 2 and 3, respectively), and matrix metalloproteinase 7 (also known as matrilysin), produced by stromal fibroblast-like cells in the vicinity of various malignancies, are suspected to have an ability to degrade components of extracellular matrix, thus promoting spread of the tumor. MMP's also have been found in epithelial tumor cells in various cancers, however, prognostic significance is not well characterized in lung cancers.

Material and methods

Microscopic slides from randomly selected cases of non-small cell lung cancer (NSCLC) from 1983 – 1994 were identified from the surgical pathology files. Tumors were graded as well differentiated (G 1), moderately differentiated (G 2) and poorly differentiated/undifferentiated (G 3/4). Tumors were staged in accordance with UICC TNM Classification, Willey-Liss, 1997. Our cases included p-Stage I to IIIA tumors. Five-micrometers thick H & E sections were reexamined. Clinical follow – up data were retrieved from the Medical Record Unit. All patients were treated by pulmonectomy or lobectomy. None of the patients received radiotherapy or chemotherapy before surgery.

These data included minimal 5-year clinical follow – up.

Formalin fixed, paraffin embedded tissue sections from 95 cases of NSCLC were immunostained with a monoclonal antibodies against MMP 3, MMP 10 and MMP 11 (NeoMarkers, Inc., Fremont, CA), using the Ventana ES automated instrument (Ventana Medical Systems, Tuscon, AZ). There were 45 SCC and 50 AC. Formalin fixed, paraffin embedded tissue sections from 99 cases of NSCLC were immunostained with a monoclonal antibodies against MMP 7 (NeoMarkers, Inc., Fremont, CA). There were 46 SCC and 53 AC. These two groups of 95 and 99 tumors contained mostly identical cases, but since research of MMP's 3, 10, 11 group and the research of MMP 7 underwent at different time periods, some cases were added to the database and some cases had to be excluded because of using up all biologic material in paraffin blocks.

Cytoplasmic immunoreactivity in the epithelial tumoral cells was semiquantitatively scored for intensity and distribution and correlated with tumor type, tumor grade, stage, tumor size, lymph node positivity, metastasis and survival. Immunoreactivity was interpreted without prior knowledge of any of the clinicopathologic parameters, separately and independently by authors. In the rare event of discrepancy, the agreement was obtained by discussion at the multiheaded scope. The intensity of staining and the distribution of cytoplasmic positivity were considered in the semiquantitative assessment of the immunohistochemical results. The distribution of staining in the tumor cells was graded as focal (< 15 %), regional (15–50 %), or diffuse (> 50 %). The intensity of cytoplasmic staining was subjectively graded as weak, moderate, or intense. Cases, in which the staining patterns were categorized as intense diffuse, intense regional, moderate diffuse, and moderate regional, were considered positive for expression for statistical analysis. Cases that were categorized as intense focal, moderate focal, and all three distributions of weak staining were considered negative for statistical analysis.

Statistical analysis

Statistical comparisons were carried out with the STATA software (Computing Resource Center, Santa Monica, CA). The chi-square test was used to determine the significance of the associations among the different variables. Disease recurrence analysis was performed with univariate models and by the Kaplan Meier method. Significance was set at $p \leq 0.05$.

Results

Cytoplasmic immunoreactivity of tumoral cells for MMP 3 (stromelysin 1) was observed in 51 % of NSCLC (in 58% of SCC and in 45 % of AC). Immunoreactivity for MMP 10 (stromelysin 2) was observed in 65 % of NSCLC (in 69% of SCC and in 62% of AC). Immunoreactivity for MMP 11 (stromelysin 3) was observed in 61% of NSCLC (in 66% of SCC and in 57 % of AC). Statistically, overexpression of MMP 10 and MMP 11 correlated with higher grade for NSCLC ($p=0.029$ and $p=0.016$, respectively), and also in a subset of AC ($p=0.015$ and $p=0.009$, respectively), but not in a subset of SCC. Also, MMP 10 and MMP 11 correlated with lymph node involvement in NSCLC ($p=0.025$ and $p=0.027$, respectively). No correlation was found for MMP 3 (stromelysin 1). Strong cytoplasmic immunoreactivity for matrilysin was observed in 43/99 (43 %) NSCLC, 24/46 (52 %) SCC and 19/53 (36 %) AC. On univariate analysis both tumor stage ($p = 0.0001$) and overexpression of MMP-7 ($p = 0.0001$) were associated with adverse clinical outcome in NSCLC. No significant correlation was found

between MMP-7 expression and tumor grade, stage, lymph node status and the presence of distant metastases. On multivariate analysis, both tumor stage (p=0. 0001) and adverse clinical outcome (p = 0.0001) were associated with overexpression of MMP 7. Overexpression of MMP-7 also independently predicted survival for the separate SCC (p = 0.003) and AC (p=0.004) tumor groups. Immunoreactivity for these MMP's was also observed in non neoplastic fibroblast-like cells in the vicinity of the tumoral mass, which was not evaluated.

Discussion

The carcinoma of the lung is the most frequent visceral malignancy worldwide, and despite some downward trends in mortality prognosis is still poor. Assessment of molecular prognostic factors, in the hope that identification of molecular risk factors will lead to individually tailored strategies, is an area of intense research.

MMP's are a group of enzymes involved in a variety of physiological and pathological processes and are thought to be responsible for both normal connective tissue matrix remodeling and accelerated breakdown associated with neoplasm development. Tissue inhibitors control the activity of MMP's. These enzymes are produced by stromal fibroblast-like cells in the vicinity of the tumor mass. Expression of various MMP's was also detected in the malignant cells themselves in various human malignancies, including pure cultures of human NSCLC cell lines (1).

The significance of this expression of MMP's by tumoral cell is uncertain. Several immunohistochemical studies have investigated the prognostic value of MMP's expression in

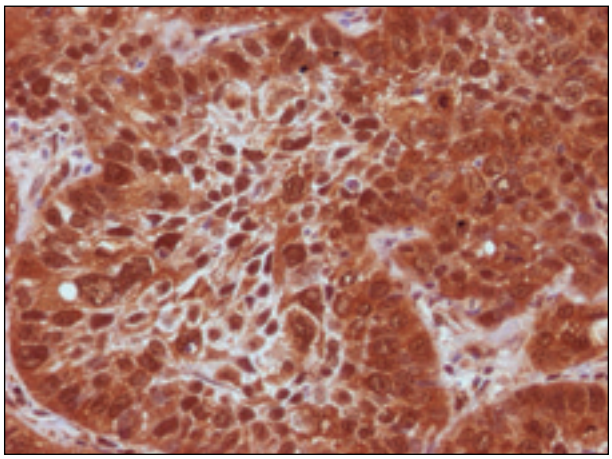

Fig. 1. Bronchogenic squamous cell carcinoma, showing intense cytoplasmic immunoreactivity for MMP 10 (stromelysin 2). (MMP 10 antibody with hematoxylin contrastain, x 200)

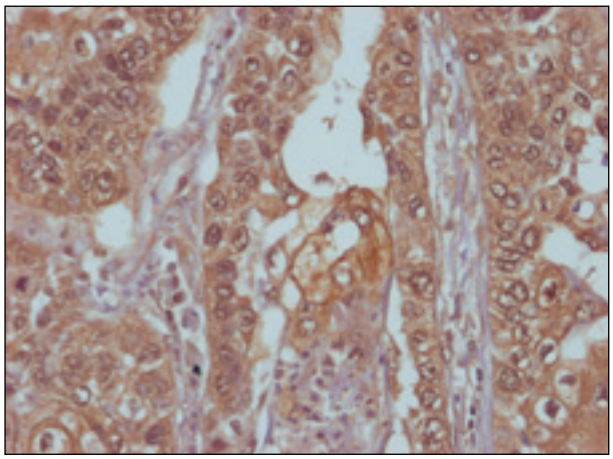

Fig. 3. Bronchogenic squamous cells carcinoma, showing intense cytoplasmic immunoreactivity for MMP 7 (matrilysin). (MMP 7 antibody with hematoxylin contrastain, x 200)

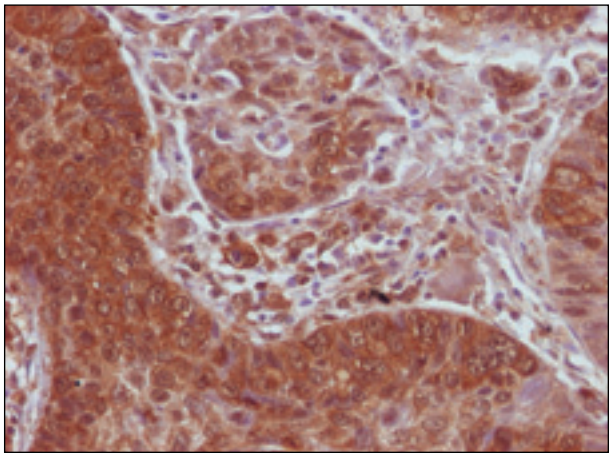

Fig. 2. Bronchogenic adenocarcinoma, showing intense cytoplasmic immunoreactivity for MMP 10 (stromelysin 2). (MMP 10 antibody with hematoxylin contrastain, x 200)

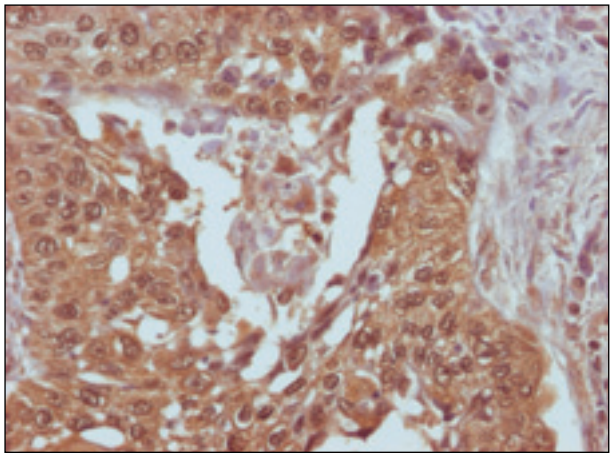

Fig. 4. Bronchogenic squamous cells carcinoma, showing intense cytoplasmic immunoreactivity for MMP 7 (matrilysin). (MMP 7 antibody with hematoxylin contrastain, x 200)

neoplastic cells in cancer, and these studies revealed inconclusive results. No correlation was found for expression of MMP 3 in our study. Expression of MMP 10 (stromelysin 2) and MMP 11 (stromelysin 3) in cytoplasm of tumoral cells is associated with higher grade in NSCLC and in AC and also is associated with lymph node involvement in NSCLC. In one study, which used transcriptional assay by quantitative RT-PCR and real-time RT-PCR analysis to validate expression of MMP 10, transcripts of MMP-10 were significantly more upregulated in recurred stage IB lung cancer than in the non-recurred stage IB lung cancer ($p = 0.004$). Also, MMP-10 monoclonal antibody showed more intense immunoreactivity in the recurred stage IB lung cancer than in the nonrecurred stage IB lung cancer ($p=0.0313$) in this study (2). With regard to the relation of expression of MMP 11 in the tumor cells and lymph node metastasis, a clear trend was observed in one study (0.0866) (3). Another study found higher expression of MMP 11 in squamous cell carcinomas with nodal metastases, which is also in accordance with our study (4). Matrilysin overexpression in NSCLC is slightly more common for SCC than AC and independently predicts shortened survival for combined NSCLC and the subset of SCC and AC. A study which used RT-PCR for detection of mRNA of MMP 7 found more elevated levels in tumor tissues from stage II-IV lung cancer compared to those from stage I lung cancer ($p = 0.0290$). There was also a tendency toward higher MMP-7 mRNA expression levels in tumors with lymph node metastasis compared to those without lymph node metastasis ($p = 0.1076$)(5). Further study of MMP-7 expression in the pathogenesis and evolution of NSCLC appears warranted.

This study suggests that, in patients who undergo surgery for NSCLC, the presence or absence of cytoplasmic immunoreactivity for MMP's could be used to define a subset of patients who may benefit from a more specific follow up and possible postoperative radiotherapy and/or intensive chemotherapy. Also, it should be worth determining different MMP expression patterns as a regimen reference for NSCLC patients who can be scheduled to receive a specific MMP inhibitor as adjuvant therapeutic agent.

Conclusion

Our data suggest that evaluation of the expression of these MMP's by tumor cells in NSCLC is useful to evaluate tumor malignancy, with possible closer follow-up and postoperative radiotherapy and/or intensive chemotherapy.

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