Histol Histopathol (1999) 14: 839-843

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Invited Review

Matrix-metalloproteinases in bronchopulmonary carcinomas

C. Martinella-Catusse[1], B. Nawrocki[1], C. Gilles[2], P. Birembaut[1] and M. Polette[1]

[1]I.N.S.E.R.M. U. 514 and Laboratoire Pol Bouin-C.H.U. de Reims, France,

[2]Laboratory of Biology of Tumours and Development-C.H.U. Sart-Tilman-Liège, Belgique

Summary. Matrix metalloproteinases (MMPs) represent a group of enzymes involved in the degradation of most of the components of the extracellular matrix and therefore participate in tumoural invasion. MMPs, especially gelatinases A and B, MT1-MMP, the activator of gelatinase A, and stromelysin-3 were found over-expressed in many cancers including bronchopulmonary carcinomas. *In vivo* observations revealed that fibroblasts are the principal source of production of MMPs. Some of these enzymes such as MT1-MMP and stromelysin 3, displayed a focal stromal localisation near preinvasive and invasive tumour clusters. Futhermore, some tumour cell lines were shown to stimulate the expression of MT1-MMP by fibroblasts. All these *in vivo* and *in vitro* results suggest that certain tumour cells produce diffusible factors which could influence the MMP stromal expression. Among these factors, the TCSF (Tumor Collagenase Stimulatory Factor) which is known to upregulate some MMPs *in vitro* could be a good candidate for this stromal regulation, since it is produced by bronchial tumour cells *in vivo*. In this review, we address such a cooperation between tumour and stromal cells for the production of MMPs and emphasize their necessity for tumoural progression in bronchopulmonary carcinomas.

Key words: Metalloproteinases, Bronchopulmonary cancer, Tumour invasion

Introduction

The metastasic progression is a multistep process including basement membrane disruption, stromal infiltration, intravasation and extravasation, and invasion of secondary organs by tumor cells. All these processes require degradation or remodeling of basement membranes and extracellular matrix macromolecules by various proteolytic enzymes. Among these proteases, matrix metalloproteinases (MMPs) are of particular interest because of their broad spectrum of substrates (Chambers and Matrisian, 1997). The MMP family consists of 4 principal subclasses, which include the collagenases, the stromelysins, the gelatinases (type IV collagenases) and the recently described group of Membrane-Type MMPs (MT-MMPs). These enzymes are secreted in a latent, proenzyme form and require activation to digest extracellular matrix components. The activation consists in a proteolytic cleavage of a propeptide domain at the N-terminus of the MMP molecule which can be accomplished by proteolytic enzymes including plasmin and other MMPs. For example, MT-MMPs participate in the activation of pro-MMP2 (progelatinase A). The tissue inhibitors of MMPs (TIMPs) specifically inhibit MMPs, and maintain a balance between matrix destruction and formation. An imbalance between MMPs and the associated TIMPs may play a significant role in the invasive phenotype of malignant tumours. Moreover, the role of MMPs is not limited to extracellular matrix degradation. Recent studies indeed implicate these enzymes in the activation of growth factors and/or their receptors (Chambers and Matrisian, 1997; Manes et al., 1997). It has now becoming clear that MMPs are implicated in a series of events leading to tumour invasion. We will focus this review on studying reported localisations of MMPs thereby identifying the cellular source of these enzymes. We will then discuss the possibility that a different pattern of MMP expression could represent different stages of metastatic progression.

MMP expression in tumoural invasion

Many culture models have been established in order to study the mechanisms involved in the tumoural development and progression of human lung cancer. From these *in vitro* systems a clear cut correlation between the acquisition of an invasive phenotype and the presence of several MMPs has immerged. Among these *in vitro* models, the human bronchial cell line 16HBE14o- derived from normal human bronchial cells immortalized with the SV-40 large T-antigen gene represent a well differentiated non-invasive stage of

Offprint requests to: Dr. Myriam Polette, I.N.S.E.R.M. U.314, 45, rue Cognacq-Jay, 51100 Reims, France

tumour progression (Cozens et al., 1994). These cells indeed showed a typical rounded epithelial phenotype, contained cytokeratins in their cytoplasm and expressed epithelial cell adhesion molecules such as E-cadherin. They did not possess invasive properties in the Boyden chamber invasion assay using a basement membrane material (Matrigel) as a barrier. They did not express any gelatinase A and MT1-MMP while they produced low amounts of gelatinase B (Yao et al., 1996; Polette et al., 1998). Three human bronchial cell lines obtained from one clone of normal human bronchial epithelial cells were tested for their invasive capacities and the expression of gelatinase A (Bonfil et al., 1989). Beas2B cells are also normal human bronchial cells infected with an adenovirus 12-SV40, non virus producers but containing the SV40 large T antigen. Unlike 16HBE14o- cells, they displayed a spindle cell pattern, with a loss of E-cadherin and expression of vimentin. Their invasive properties in Matrigel were, however, limited and accordingly they produced low amounts of gelatinase A. BZR cells were developed from Beas2B cells infected with recombinant virus containing the v-Ha-ras oncogene. BZRT33 cells were derived from a tumour formed by BZR cells injected s.c. into an athymic nude mouse. Both the BZR and the BZRT33 cell lines displayed a spindle shape, cellular expansions (pseudopodia and lamellipodia). Their cytoskeleton contained intermediate filaments of vimentin whereas E-cadherin was undetectable (Polette et al., 1998). These cells were highly invasive in the Boyden chamber assay and they expressed both gelatinase A and MT1-MMP (Ura et al., 1989; Polette et al., 1998). These different cell lines therefore clearly represent different stages of progression towards the invasive phenotype. The invasive phenotype appears well associated with the expression of mesenchymal characteristics by transformed epithelial cells and with the production of MMPs.

The expression of different MMPs and their inhibitors has been well documented in various series of lung cancers. Gelatinase A (Urbanski et al., 1992;

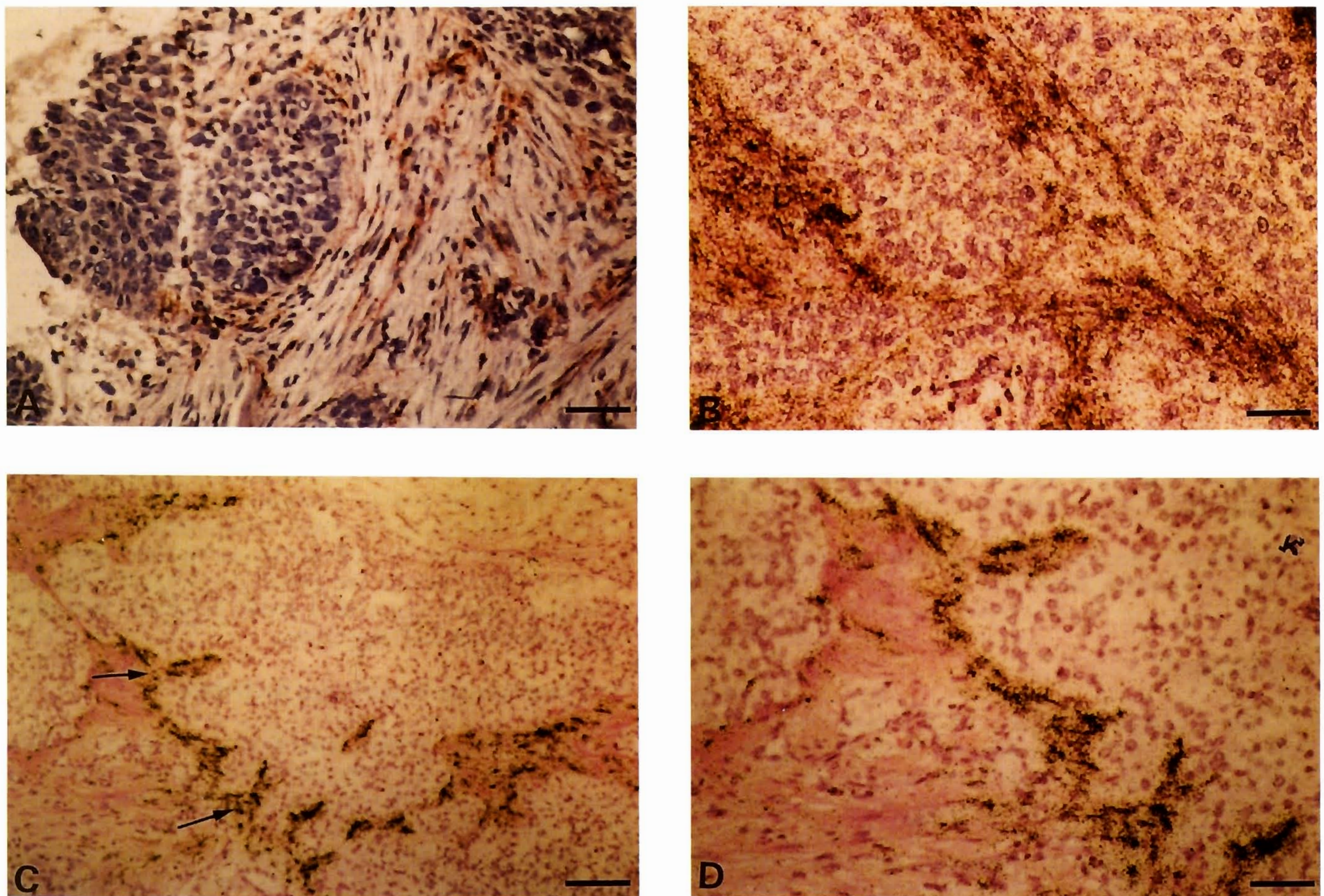

Fig. 1. Gelatinase A is localised by immunohistochemistry **(A)** and by in situ hybridisation **(B)** in fibroblasts surrounding invasive tumour cells of bronchopulmonary carcinoma (Bar: 70 μm). **C.** MT1-MMP mRNAs are expressed in some fibroblasts (arrow) near tumour clusters of bronchopulmonary carcinoma (Bar: 120 μm). **D.** Higher magnification of the same area showing that the MT1-MMP-positive fibroblasts are in close contact with tumour cells (Bar: 70 μm).

Brown et al., 1993; Soini et al., 1993; Kawano et al., 1997), gelatinase B (Urbanski et al., 1992; Canete-Soler et al., 1994), MT1-MMP (Tokuraku et al., 1995; Polette et al., 1996), stromelysin 3 (Urbanski et al., 1992; Anderson et al., 1995; Bolon et al., 1996, 1997), collagenase 1 (Muller et al., 1991; Urbanski et al., 1992; Bolon et al., 1995), stromelysin 1 (Muller et al., 1991; Bolon et al., 1997) and matrilysin (Muller et al., 1991; Urbanski et al., 1992; Bolon et al., 1997; Kawano et al., 1997) have been detected in different histological types of bronchopulmonary carcinomas by various approaches: immunohistochemistry, *in situ* hybridization, Northern blot and zymography. Studying 90 pulmonary bronchopulmonary carcinomas by Northern blot analysis, we observed that MMP expression frequencies and mRNA levels increased progressively with malignant phenotype, lack of differentiation and TNM stage of the tumours, whereas TIMP1 and TIMP2 expression decreased very early during tumour progression (Nawrocki et al., 1997). We also found the expression of all these MMPs in all of our tumours. In agreement with the literature, the predominantly expressed MMPs were gelatinase A, MT1-MMP and stromelysin 3 which were significantly co-expressed suggesting their co-regulation in these carcinomas. All these results indicate that tumour progression in bronchopulmonary carcinomas implies a progressive disruption of the MMP/TIMP balance leading to an excess of several degradative MMPs, essentially gelatinase A, MT1-MMP and stromelysin 3, that act in concert *in vivo*.

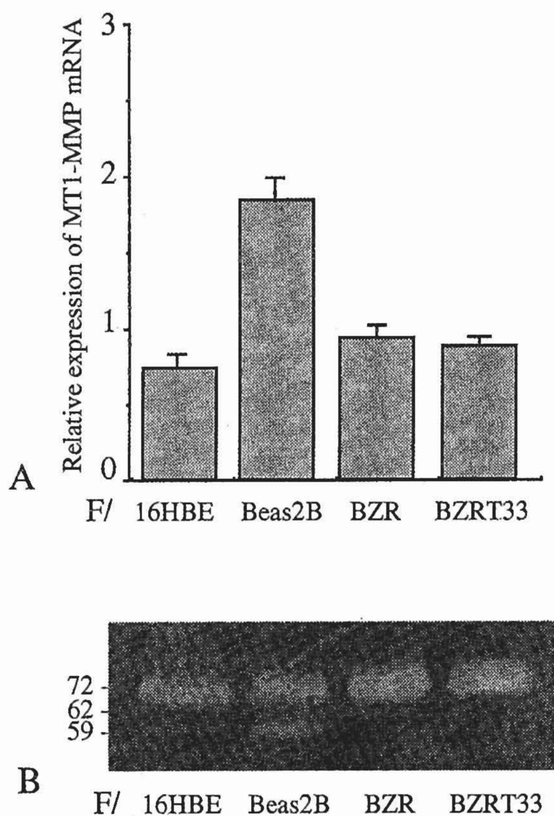

Fig. 2. A. Histogram of the relative expression of MT1-MMP mRNAs. The relative amounts are expressed as mean±SEM. Fibroblasts (F) treated with conditioned media of 16HBE14o- (16HBE): 0.74±0.1, Beas2B: 1.86±0.12, BZR: 0.95±0.8 and BZRT33: 0.84±0.06. **B.** Gelatinolytic activities in the conditioned media of fibroblasts treated with conditioned media of 16HBE14o- (16HBE), Beas2B, BZR and BZRT33. The position of the different forms of gelatinase A (in kilodalton) are indicated.

Our morphological studies using *in situ* hybridization and immunohistochemistry revealed the expression of MMPs and TIMPs mostly in stromal cells in close contact with tumour clusters. Stromal cells involved in the production of most MMPs (gelatinase A, gelatinase B, MT1-MMP, stromelysins, collagenase) were principally fibroblasts (Fig. 1). Some enzymes, particularly stromelysin-3 and MT1-MMP displayed a focal stromal expression near some invasive and pre-invasive tumour cells (Fig. 1). Moreover, stromelysin-3 expression by fibroblasts has also been detected in precancerous lesions of the lung (Bolon et al., 1996). Tumour cells rarely produced MMPs except matrilysin and MT1-MMP (Bolon et al., 1995, 1997). In addition, these latter authors have reported the expression of stromelysin 3 by some cancer cells in 24% of their tumours, especially in bronchial dysplasias and *in situ* carcinomas, a finding which was not observed by us nor Anderson et al. (1995). Some inflammatory cells like macrophages have also been described as a source of gelatinase B (Canete-Soler et al., 1994). All these data support the important role of stromal cells in the extra-cellular matrix remodeling and the close cooperation between these host cells and cancer cells in tumour invasion and metastasis in bronchopulmonary carcinomas.

MMPs and cooperation between tumour and stromal cells

Since our *in vivo* results suggested that fibroblasts could be influenced by certain tumour cells to produce MMPs, we used several bronchial cell lines (16HBE14o-, Beas2B, BZR and BZRT33) previously characterized for their invasiveness, phenotype and expression of MMPs to investigate this hypothesis. Human dermal fibroblasts were cultured in presence of conditioned media from these different bronchial cell lines. The production of gelatinase A and MT1-MMP was compared to the basal production of MMPs by these fibroblasts. Conditioned medium from 16HBE14o-cells, as well as those from BZR and BZRT33 cells, did not increase the basal expression of MT1-MMP by fibroblasts (Fig. 2). In contrast, conditioned medium from Beas2B cells significantly stimulated the production of MT1-MMP by fibroblasts, with a subsequent activation of progelatinase A (Fig. 2). Thus, these poorly invasive cells were the most potent to cooperate with fibroblasts for the stimulation of production of MMPs *in vitro*.

All these results emphasize the existence of a

tumoural soluble factor which influences the stromal expression of MMPs. For instance, transforming-growth factor ß (TGF-ß) has been shown to induce the synthesis of gelatinase A (Salo et al., 1991). This factor was also detecteded by both non-small cell lung carcinoma and Beas2B cell lines suggesting that it may be a good candidate for the stimulation of pulmonary fibroblasts by tumour cells *in vivo* (Bergh, 1990; Linnala et al., 1995). Since a few years ago, another tumoural factor, the TCS-F (Tumor Collagenase Stimulatory Factor), also named EMMPRIN (Extracellular Matrix MetalloProteinase Inducer) is considered to be molecule potentialy involved in the cooperation between cancer cells and fibroblasts. It was isolated from the plasma membranes of a human lung carcinoma cell line (Ellis et al., 1989). This glycoprotein, a member of the Ig superfamily, was detected on the outer surface of human carcinoma cells but it was not observed in most normal cells *in vitro* nor *in vivo*. TCS-F stimulated fibroblasts to produce elevated levels of collagenase 1 (MMP-1), gelatinase A and stromelysin 1 (MMP-3) (Kataoka et al., 1993, Guo et al., 1997). The plasma membrane localization of TCS-F may serve to restrict its bioactivity to cells in close proximity. However, tumour cells may also shed TCS-F as components of membrane vesicles, thereby enabling TCS-F activity to extend beyond the direct cell contact effect. To explore the eventual role of TCS-F *in vivo* in bronchopulmonary carcinomas, we detected by *in situ* hybridization and immunohistochemistry its expression in various tumours and normal tissues. By *in situ* hybridization, we showed that most inavasive and preinvasive tumour cells express mRNAs encoding TCS-F with a concomitant expression of gelatinase A in adjacent stromal cells on serial sections. The adjacent normal bronchial epithelial cells did not express any TCS-F transcripts. However, no significant differences between the TCS-F mRNA levels quantified by Northern blot analysis were found in accordance with the TNM stage (Polette et al., 1997). Immunohistochemical detection of TCS-F showed the presence of this factor in most tumours, except neuroendocrine carcinomas. TCS-F was localized on the cell surface of all tumour cells and in normal epithelial cells. This latter observation may correspond to a low basal level of expression of TCS-F mRNAs in normal tissues undetectable by *in situ* hybridization. Moreover, some stromal fibroblasts and endothelial cells were also stained, indicating the possibility of TCS-F receptors on these cells. Our studies show that TCS-F, even if it cannot be considered as a prognosis factor, intervenes in the progression of bronchopulmonary carcinomas *in vivo*.

Conclusion

MMPs were overexpressed in bronchopulmonary carcinomas and were principally found in fibroblasts in close contact to the invasive and preinvasive tumour clusters. The *in vivo* and *in vitro* data suggest an elective cooperation between tumour and stromal cells. Accordingly, the non invasive 16HBE14o- cells and the most invasive tumour BZR and BZRT33 cells were not able to stimulate fibroblasts for the production of gelatinase A and MT1-MMP while the weakly invasive Beas2B cells could. The invasive tumour cells producing their own proteolytic enzymes could represent *in vivo* a limited population of metastatic cancer cells which would not require the contribution of stromal MMPs to be able to disseminate. In contrast, poorly invasive cells (Beas2B) may be the *in vitro* equivalent of most preinvasive cancer cells which do not produce most of MMPs and need the active participation of fibroblasts to invade the stroma *in vivo*. More than representing distinct processes of tumour invasion, these different cooperative schemes between tumour and stromal cells could also reflect the different stages of tumoural invasion. The identification of the MMP stimulating factors may be a further target to hamper tumour progression.

Acknowledgements. To the LIONS Club of Soissons and Villers-Cotterets for their support of this work.

References

Anderson I.C., Sugarbaker D.J., Ganju R.K., Tsarwhas D.J., Richards W.G., Sunday M., Kobzik L. and Shipp M.A. (1995). Stromelysin 3 is overexpressed by stromal elements in primary non small cell lung cancers and is regulated by retinoic acid in pulmonary fibroblasts. Cancer Res. 55, 4120-4126.

Bergh J.C.S. (1990). Gene amplification in human lung cancer. The *myc*-genes and other protooncogenes and growth factor genes. Am. Rev. Resp. Dis. 142, S20-S26.

Bolon I., Gouyer V., Devouassoux M., Vandenbunder B., Wernert N., Moro D., Brambilla C. and Brambilla E. (1995). Expression of c-ets-1, collagenase 1 and urokinase-type plasminogen activator genes in lung carcinomas. Am. J. Pathol. 147, 1298-1310.

Bolon I., Brambilla E., Vandenbunder B., Lanteejoul S., Robert C. and Brambilla C. (1996). Changes in the expression of matrix metalloproteases and of the transcription factor c-Ets-1 during progression of precancerous bronchial lesions. Lab. Invest. 75, 1-13.

Bolon I., Devouassoux M., Robert C., Moro D., Brambilla C. and Brambilla E. (1997). Expression of urokinase-type plasminogen activator, stromelysin 1, stromelysin 3 and matrilysin genes in lung carcinomas. Am. J. Pathol. 150, 1619-1629.

Bonfil R.D., Reddel R.R., Ura H., Reich R., Fridman R., Harris C.C. and Klein-Szanto A.J.P. (1989). Invasive and metastatic potential of a v-Ha-ras-transformed human bronchial epithelial cell line. J. Natl. Cancer Inst. 81, 587-594.

Brown P.D., Bloxidge R.E., Stuart N.S.A., Gatter K.C. and Carmichael J. (1993). Association between expression of activated 72-kilodalton gelatinase and tumor spread in non-small-cell lung carcinoma. J. Natl. Cancer Inst. 85, 574-578.

Canete-Soler R., Litsky L., Lubensky I. and Muschel R. (1994). Localization of the 92 kD gelatinase mRNA in squamous cell and adenocarcinomas of the lung using *in situ* hybridization. Am. J. Pathol. 144, 518-527.

Chambers A.F. and Matrisian L.M. (1997). Changing views of the role of matrix metalloproteinases in metastasis. J. Natl. Cancer Inst. 89, 1260-1270.

Cozens A.L., Yezzi M.J., Reich R., Kunzelmann K., Ohrui T., Chin L., Eng K., Finkbeiner W.E., Widdicombe J.H. and Gruenert D.C. (1994). CFTR expression and chloride secretion in polarized immortalized human bronchial epithelial cells. Am. J. Respir. Cell Mol. Biol. 10, 38-47.

Ellis S.M., Nabeshima K. and Biswas C. (1989). Monoclonal antibody preparation and purification of a tumor cell collagenase-stimulatory factor. Cancer Res. 49, 3385-3391.

Guo H., Zucker S., Gordon M.K., Toole B.P. and Biswas C. (1997). Stimulation of matrix metalloproteinase production by recombinant extracellular matrix metalloproteinase inducer from transfected Chinese hamster ovary cells. J. Biol. Chem. 272, 24-27.

Kataoka H., DeCastro R., Zucker S. and Biswas C. (1993). Tumor cell-derived collagenase-stimulatory factor increases expression of interstitial collagenase, stromelysin, and 72-kDa gelatinase. Cancer Res. 53, 3154-3158.

Kawano N., Osawa H., Ito T., Nagashima Y., Hirahara F., Inayama Y., Nakatani Y., Kimura S., Kitajima H., Koshikawa N., Miyazaki K. and Kitamura H. (1997). Expression of gelatinase A, tissue inhibitor of metalloproteinase-2, matrilysin and trypsin(ogen) in lung neoplasms: an immunohistochemical study. Hum. Pathol. 28, 613-622.

Linnala A., Kinnula V., Laitinen L.A., Lehto V.P. and Virtanen I. (1995). Transforming growth factor-ß regulates the expression of fibronectin and tenascin in Beas2B human bronchial epithelial cells. Am. J. Respir. Cell Mol. Biol. 13, 578-585.

Manes S., Mira E., Barbacid M.D.M., Ciprés A., Fernandez-Resa P., Buesa J.M., Mérida I., Aracil M., Marquez G. and Martinez-A C. (1997). Identification of insulin-like growth factor-binding protein-1 as a potential physiological substrate for human stromelysin 3. J. Biol. Chem. 272, 25706-25712.

Muller D., Breatnach R., Engelmann A., Millon R., Bronner G., Flesh H., Dumont P., Eber M. and Abecassis J. (1991). Expression of collagenase-related metalloproteinase genes in human lung or head and neck tumours. Int. J. Cancer 48, 550-556.

Nawrocki B., Polette M., Marchand V., Monteau M., Gillery P., Tournier J.M. and Birembaut P. (1997). Expression of matrix metalloproteinases and their inhibitors in human bronchopulmonary carcinomas: quantitative and morphological analyses. Int. J. Cancer 72, 556-564.

Polette M., Nawrocki B., Gilles C., Sato H., Seiki M., Tournier J.M. and Birembaut P. (1996). MT-MMP expression and localisation in human lung and breast cancers. Virchows Arch. 428, 29-36.

Polette M., Gilles C., Marchand V., Lorenzato M., Toole B., Tournier J.M., Zucker S. and Birembaut P. (1997). Tumour Collagenase Stimularoty Factor (TCSF) expression and localisation in human lung and breast cancers. J. Histochem. Cytochem. 45, 703-710.

Polette M., Gilles C., de Bentzmann S., Gruenert D., Tournier J.M. and Birembaut P. (1998). Association of fibroblastoid features with the invasive phenotype in human bronchial cancer cell lines. Clin. Exp. Metastasis 16, 105-112.

Salo T., Lyons J.G., Rahemtula F., Birkedal-Hansen H. and Larjava H. (1991). Transforming growth factor ß1 upregulates type IV collagenase expression in cultured human keratinocytes. J. Biol. Chem. 266, 11436-11441.

Soini Y., Pääkkö P. and Autio-Harmainen H. (1993). Genes of laminin ß1 chain, α1(IV) chain of type IV collagen, and 72 kD type IV collagenase are mainly expressed by the stromal cells of lung carcinomas. Am. J. Pathol. 142, 1622-1630.

Tokuraku M., Sato H., Murakami S., Okada Y., Watanabe Y. and Seiki M. (1995). Activation of the precursor of gelatinase A/ 72 kDa type IV collagenase/ MMP-2 in lung carcinomas correlates with the expression of membrane-type matrix metalloproteinase (MT-MMP) and with lymph node metastasis. Int. J. Cancer 64, 355-359.

Ura H., Bonfil R.D., Reich R., Reddel R., Pfeifer A., Harris C.C. and Klein-Szanto A.J.P. (1989). Expression of type IV collagenase and procollagenase genes and its correlation with the tumorigenic, invasive, and metastatic abilities of oncogene-transformed human bronchial epithelial cells. Cancer Res. 49, 4615-4621.

Urbanski S.J., Edwards D.R., Maitland A., Leco K.J., Watson A. and Kossakowska A.E. (1992). Expression of metalloproteinases and their inhibitors in primary pulmonary carcinomas. Br. J. Cancer 66, 1188-1194.

Yao P.M., Bulher J.M., d'Ortho M.P., Lebargy F., Delclaux C., Harf A. and Lafuma C. (1996). Expression of matrix metalloproteinase gelatinases A and B by cultured epithelial cells from human bronchial explants. J. Biol. Chem. 271, 15580-15589.

Accepted November 19, 1998