Invited Review

Matrix-metalloproteinases in bronchopulmonary carcinomas

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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 overexpressed 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. Furthermore, 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 metastatic progression is a multistep process including basement membrane disruption, stromal infiltration, intravasation and extravasation, and invasion of secondary organs by tumour 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...
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. BZR-T33 cells were derived from a tumour formed by BZR cells injected s.c. into an athymic nude mouse. Both the BZR and the BZR-T33 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;
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 (Tokaruku et al., 1995; Polette et al., 1996), stromelysin 3 (Urbanski et al., 1992; Anderson et al., 1995; Bolon et al., 1996, 1997), collagenase I (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.

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 extracellular 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 β (TGF-β) has been shown to induce the synthesis of gelatinase A (Sato et al., 1991). This factor was also detected 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 potentially 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 A (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 invasive 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 (Poletti 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 tumour invasion. The identification of the MMP stimulating factors may be a further target to hamper tumour progression.

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References


MMPs and lung carcinomas


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