XIXth MEETING

of the Federation of the

European Connective Tissue Societies

Programme and Abstracts

Taormina - Giardini Naxos, Italy

9 - 13 July 2004

ORAL PRESENTATION - WORKSHOP "F"

OF1

TYPE II COLLAGEN FRAGMENTS AS ACTIVATORS OF METALLOPROTEASE EXPRESSION IN CHONDROCYTES

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Introduction – Collagenous degradation products are typical constituents of normal and degenerative cartilage and may contribute to regulate matrix biosynthesis. Here we present evidence for the induction of various matrix metalloproteases (MMP) by collagenase-generated collagen fragments (colf) and by a specific synthetic peptide modeled from the N-terminal telopeptide (N-telo), at concentrations even below those found in diseased cartilage (up to 20% of total collagen).
Methods – Isolated bovine chondrocytes and bovine or human cartilage explants were treated for up to three weeks with various doses of the collagen type II peptides in a synthetic culture medium. The medium was then tested for the presence of MMPs by gelatin zymography and Western blotting. Expression levels for MMP mRNA were measured by Light Cycler PCR. MMP-3 levels in medium were quantified by ELISA. In explants, proteoglycan content was defined by dimethylenblue assay, collagen via hydroxyprolin determination.
Results and Discussion – Significant upregulation (4 to 500fold) of message was found for MMP-2, MMP-3, MMP-9, and MMP-13, depending on MMP and fragment dose in a dose range from 0.1 to 10 mg/ml. Zymography revealed both up-regulation and activation of pro-MMP-2 and –9. Cartilage explants experienced various degrees of proteoglycan losses, little loss of collagen. Taken together, the incubation of chondrocytes or cartilage explants with even moderate amounts of fragmented collagen molecules seems to induce a vicious circle of matrix degradation triggered by a relatively unspecific activation of MMP expression.

OF2

ADAM12 IS SELECTIVELY OVEREXPRESSED IN HUMAN GLIOBLASTOMAS AND IS ASSOCIATED WITH GLIOBLASTOMA CELL PROLIFERATION AND SHEDDING OF HB-EGF

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[Introduction] ADAMs (a disintegrin and metalloproteinases) are multifunctional molecules involved in cell-cell fusion, cell adhesion, membrane protein shedding and proteolysis. The aim in the present study was to identify the ADAM species overexpressed in human glioblastomas and study its pathobiological function in the tumor. [Methods] mRNA expression of 13 different ADAM species with putative metalloproteinase activity was screened in human astrocytic tumors, non-neoplastic brain tissues and other intracranial tumors by RT-PCR, and the levels were measured by real-time quantitative PCR. The cells responsible for the expression and production were examined by in situ hybridization and immunohistochemistry. Protein expression was also determined by immunoblotting. Implication of ADAM12 for shedding of the precursor of heparin-binding epidermal growth factor (HB-EGF) was analyzed by immunoblotting. [Results and Discussion] Among the ADAM species examined, prototype membrane-anchored ADAM12 (ADAM12m) was predominantly expressed in glioblastomas. The expression level of ADAM12m was remarkably at least 5.7-fold higher in glioblastomas than in non-neoplastic brain tissues, low grade and anaplastic astrocytic tumors ($p < 0.05$ for each group) and intracranial neurinomas ($p < 0.01$). In situ hybridization showed that glioblastoma cells are responsible for the gene expression. ADAM12m was predominantly immunolocalized on the cell membrane of glioblastoma cells. Immunoblotting analysis demonstrated that ADAM12m is expressed as an activated N-glycosylated form of ~90 kDa in glioblastoma tissues. A direct correlation was seen between the mRNA expression levels of ADAM12m and proliferative activity of gliomas ($r = 0.791$, $p < 0.0001$; $n = 32$). Shedding of HB-EGF, a substrate of ADAM12m, was observed by immunoblotting in glioblastoma samples showing a correlation with the ADAM12m expression, and inhibited by treatment with ADAM inhibitor of the glioblastomas. These data demonstrate that ADAM12m is highly expressed in human glioblastomas, and suggest the possibility that ADAM12m plays a role in the prominent proliferation of the glioblastomas through shedding of HB-EGF.

OF3

EXPRESSION OF ADAMTS -1, -4 -5, TIMP-3 AND VERSICAN IN MULTIPLE SCLEROSIS CNS TISSUE.

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Introduction
In multiple sclerosis (MS) complex changes occur in the CNS extracellular matrix (ECM) during lesion development. CNS chondroitin sulphate proteoglycans (CSPGs) versican, aggrecan, brevican and neurocan promote cell adhesion, migration and influence neurite outgrowth (1). ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) enzymes are a relatively new group of metalloproteinases (2). The ECM substrates degraded by ADAMTS-1, -4 and -5, including CSPGs, suggest that they play a role in turnover of the CNS ECM. TIMP-3 is the major inhibitor of ADAMTSs but heparin, chondroitin sulphate and thrombospondins also inhibit aggrecanase activity. ADAMTSs and TIMP-3, in MS, may modulate ECM breakdown as well as affecting demyelination and prevention of remyelination. Thus the potential role of these enzymes in MS has been investigated.
Methods
CNS tissue from MS and controls (UK MS Tissue Bank, Charing Cross Hospital, London, UK) was assessed for the expression of ADAMTS-1, -4 and -5, TIMP-3 and the ECM component, versican, at the mRNA and protein level by qRT-PCR, western blotting and immunocytochemistry.
Results and Discussion
ADAMTS -1, -4 and -5, versican and TIMP 3 were all expressed at the mRNA and protein level in control and MS tissue. The levels of ADAMTSs, versican and TIMP-3 differ in MS compared to control CNS tissue. This provides the first report of modulation of ADAMTS expression in MS tissue.
References
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OF4

ZYMOGRAPHIC DETECTION OF MATRIX PROTEASES IN BREAST CANCER BIOPSIES BY MONO- AND TWO-DIMENSIONAL ELECTROPHORESIS

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Introduction
Matrix metalloproteinases (MMPs) have received great attention in recent years as putative tumor markers for clinical applications. Indeed, current evidence suggests that several members of the MMP family, rather than simply degrading ECM, perform multiple functions, including the mobilization of growth factors and processing of surface molecules. The matrix metalloproteinases are the major, but not the only enzymes involved in the process of tumor invasion: other families of extracellular enzymes, such as serine and cystine proteinases are directly or indirectly responsible for the ECM degradation during invasion and metastasis. In the attempt to recognize an enzymatic signature for breast cancer progression, we used different substrates, namely gelatin, casein and C-methylated transferrin to study the mono- and 2D-zymographic pattern (1,2) of breast cancer biopsy fragments.
Methods: mono-dimensional and two-dimensional zymography, western blot, ion exchange and affinity chromatography
Results and Discussion
Zymographic assays performed by mono-dimensional electrophoresis showed higher activity levels of MMP-2 and MMP-9 in the tumor samples with respect to the adjacent normal tissue. Moreover tumor breast tissue extracts showed the exclusive presence of activated forms for MMP-2 in contrast with non-tumoral counterparts. More interestingly, the comparative analyses of 2D-zymographic assays on specific substrates (gelatin, casein and C-methylated transferrin) revealed a peculiar enzymatic pattern of MMPs and serine proteases as compared with the non-tumoral counterparts.
Reference
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Work supported by MIUR to I.PM.