DETECTION OF MT1-MMP IN FIBROBLASTS AND OSTEOCLASTS AT BONE RESORPTION SITES OF THE BONE-IMPLANT INTERFACE AROUND LOOSE JOINT ARTHROPLASTIES BY IN-SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY

Introduction 
It has been understood that the aseptic loosening of joint arthroplasties is an active process which is linked to the development of a synovial-like membrane at the bone-implant interface. Formation of this interface membrane is widely considered a foreign body reaction initiated by wear debris particles from the prosthesis. However, the cellular mechanisms of membrane formation and consecutive prosthesis loosening are not fully understood. Recent data suggest that matrix metalloproteinases (MMP's) contribute significantly to matrix degradation responsible for the loosening of failed joint prostheses. Although several studies have reported MMP expression in osteoclasts recently, there has been no clear description of MMP-mediated bone resorption in prosthesis loosening. Furthermore, the role of newly found membrane-type metalloproteinases (MT-MMP's) as well as their expression pattern in different cell types needs still to be established. One of these MT-MMP's, MT1-MMP, is of special interest, because it is known to exert dual activity: MT1-MMP is able to degrade different extracellular matrix components as well as to activate proMMP2 and proMMP13. Here, we investigated the mRNA and protein expression of MT1-MMP as well as its distribution pattern within the bone-implant interface of aseptically loosened joint arthroplasties. In addition, MMP2 activity was determined.

Methods 
Samples of the interface membrane around loose cementless joint arthroplasties which contained both fibrous tissue and attached bone were obtained from 2 patients with failed prosthetic knee and 4 patients with failed prosthetic hip joints at revision surgery. Specimens were embedded in paraffin and 5 µm sections were analyzed using hematoxylin and eosin staining as well as by applying monoclonal anti-CD68 antibodies (Clone PG-M1, Dako) in an APAAP technique. Immunohistochemistry using a MT1-MMP specific antibody (RDI-MMP14Habr, Research Diagnostics, Flanders NJ, USA) was then used on serial sections to analyze the MT1-MMP expression on mRNA level. In addition, we performed zymography to determine the activity of MMP-2, one of the MT1-MMP substrates, within the synovial-like membranes.

Results: 
Our studies revealed the presence of MT1-MMP both on mRNA and on protein level within all interface samples investigated (Fig. 1a). There was a distinct expression pattern of MT1-MMP with negligible staining in those areas of the synovial-like membranes originally located next to the prosthesis but abundant staining at sites attached to bone (Fig 1b). Apart from being present in mononucleated cells of fibroblast-like shape, MT1-MMP mRNA as well as protein was found expressed highly in multinucleated cells at the very site of bone resorption, which morphologically appeared to be osteoclasts (Fig 1a). Double labeling using immunohistochemistry with monoclonal anti-CD68 antibodies revealed positive staining of these multinucleated cells but almost no staining of other MT1-MMP positive cells at the bone resorption area confirming their identities as osteoclasts and fibroblasts. By zymography considerable MMP-2 activity could be observed within the synovial-like membranes

Discussion: 
Investigating paraffin sections of interface membranes around loose joint arthroplasties which contained fibrous tissue as well as the bone to which this tissue was attached, we were able to evaluate the direct site of bone resorption. We could demonstrate that MT1-MMP, which to date has only been reported in rat osteoclasts, is present in human osteoclast in situ and is abundantly expressed in such cells at the site of bone resorption of failed prosthetic joints. Interestingly, MT1-MMP could not only been demonstrated on mRNA but also on protein level in these cells. MT1-MMP was also found in fibroblasts at sites of bone resorption. The expression pattern of MT1-MMP, with only limited staining at the site of the prosthesis, but high expression at the site of bone resorption, is of particular interest. It may point to a differentiation not only of macrophages into osteoclasts but also of fibroblast-like cells towards more aggressive behavior. These findings support previous studies which have demonstrated the activation of human fibroblasts by wear debris particles. Moreover, the presence of MT1-MMP in osteoclasts and fibroblasts as well as the zymography results also suggest, that this protease may not only play a role in osteoclast-mediated bone resorption in prosthesis loosening but also contribute to degradation of extracellular matrix by activating MMP-2 from fibroblasts.

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