Phagocytosis of collagen by fibroblasts and invasive cancer cells is mediated by MT1-MMP

H. Lee*, K.L. Sodek‡, Q. Hwang*, T.J. Brown‡, M. Ringuette† and J. Sodek*†

*CIHR Group in Matrix Dynamics, Faculty of Dentistry, Room 234, Fitzgerald Building, University of Toronto, 150 College Street, Toronto, ON, Canada M5S 3E2, ‡Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada M5S 3G5, and †Department of Obstetrics and Gynaecology, University of Toronto, Toronto, ON, Canada, M5G 1L4

Abstract
Degradation of collagen is required for the physiological remodelling of connective tissues during growth and development, as well as in wound healing, inflammatory diseases, and cancer cell invasion. In remodelling adult tissues, degradation of collagen occurs primarily through a phagocytic pathway. While various steps in this pathway have been characterized, the enzyme required to fragment collagen fibrils for phagocytosis has not been identified. Laser confocal microscopy, transmission electron microscopy and biochemical assays were used to show that degradation of collagen substrates by fibroblasts correlated with the expression of the membrane-bound metalloproteinase MT1-MMP (membrane-type 1 matrix metalloproteinase). The MT1-MMP was localized to sites of collagen cleavage on the cell surface and also within the cells. In contrast with MT1-MMP, the gelatinase MMP-2 was not required for collagen phagocytosis. Similar analyses of several ovarian cancer, breast cancer and fibrosarcoma cells indicated that highly metastatic cells also degrade collagen through a phagocytic pathway that is mediated by MT1-MMP. Collectively, these studies demonstrate a pivotal role for catalytically active MT1-MMP in preparing collagen fibrils for phagocytic degradation by normal and transformed cells.

Introduction
Degradation of collagen is a fundamentally important process in the growth and development of connective tissues, maintenance of tissue homoeostasis in mature tissues and in wound healing. In various inflammatory diseases, excessive collagen degradation by fibroblasts leads to tissue destruction whereas reduced activity results in scar formation and fibrosis. Moreover, the ability of cancer cells to degrade fibrillar collagens is required for tumour growth and metastasis. Although collagen degradation by metalloproteinases is considered to occur extracellularly, previous studies have shown that in rapidly remodelling mature connective tissues, degradation occurs through an intracellular phagocytic pathway [1,2]. While various steps in the phagocytic process have been characterized [3], the enzyme responsible for the requisite fragmentation of collagen fibrils has only recently been identified as the MT1-MMP (membrane-type 1 matrix metalloproteinase) [4]. Although MT1-MMP is a potent collagenase, it also acts on other matrix and cell surface-associated proteins and can activate pro-MMP-2, a gelatinase that can degrade stromal and basement membrane barriers [5,6]. While studies of knockout mice have demonstrated the importance of MT1-MMP in matrix remodelling [7], expression of MT1-MMP has also been associated with the aggressive behaviour of cancer cells [8] and shown to have a critical role in cancer cell invasion of collagen matrices [9,10]. Studies indicate that MT1-MMP is translocated to the leading edge of invasive cancer cells, where its pericellular proteolytic activity can be focused [11]. However, it is not known whether the subsequent degradation of collagen occurs extracellularly or involves phagocytosis and intracellular degradation. To address this question, we have used a combination of morphological and biochemical approaches to study collagen degradation by several cancer cells.

Results
For these studies a fibrosarcoma cell line (HT1080) and several breast (MDA-MB-231, MCF-7 and T47D) and ovarian cancer cell lines (HEY, SKOV-3 and OVCAR-3) were analysed for their ability to phagocytose collagen. The degradation of type I collagen was first determined using biotin-labelled reconstituted and natural collagen substrates and compared with normal HGFs (human gingival fibroblasts), which have been shown to degrade collagen through a phagocytic pathway [4]. Whereas all of the cancer cells were able to degrade collagen, only the more aggressive cancer cell lines including the HT1080 and MDA-MB-231 cells and ConA (concanavalin A)-stimulated HGFs expressed appreciable levels of MT1-MMP, as determined by real-time RT (reverse transcription)–PCR, Western blotting, and immunostaining (Figure 1). That collagen degradation was mediated by collagenase activity was evident from the immunostaining observed with an antibody that recognizes the neoepitope that is generated on the COLI 3/4-fragment (Figure 1B). Staining was evident in the vicinity of HGFs and cancer cells that were degrading the collagen substratum. That MT1-MMP was required for cancer cell invasion was...
Figure 1 | Analysis of MT1-MMP expression and activity

(A) Western blot for MT1-MMP shows expression (*) in MDA-MB-231, HT1080 and ConA-stimulated HGF (lanes 1, 4 and 5). Although a closely migrating band is seen in the poorly invasive MCF-7 and T47D cells (lanes 2 and 3), no RNA transcript was detected by real-time RT–PCR (values shown on gel are relative to HGF = 1.0). (B) HGF and HEY cells were cultured on biotinylated reconstituted rat tail collagen for 48 h and fluorescently stained without permeabilization for F-actin with phalloidin–Alexa Fluor 488 (green), for MT1-MMP with AB815 (rabbit anti-human MT1-MMP hinge) or rabbit anti-COLI (collagen type I) 3/4 fragment followed by goat anti-rabbit Alexa Fluor 594 (red), and for biotinylated collagen with streptavidin–Alexa Fluor 633 (blue), for MT1-MMP with AB815 (rabbit anti-human MT1-MMP hinge) or rabbit anti-COLI (collagen type I) 3/4 fragment followed by goat anti-rabbit Alexa Fluor 594 (red), and for biotinylated collagen (green). Note the appearance of collagen fragments (yellow arrows) above the substratum associated with the actin in the plane of the cell.

Figure 2 | Evidence of intracellular collagen degradation

(A) Increased numbers of lysosomes, stained with LysoTracker Red DND-99 in MDA-MB-231 cells became stained with phalloidin–Alexa Fluor 633 (blue) when degrading biotinylated collagen (green) compared with the same cells on a fibronectin substratum. (B) Confocal images in the X–Y and Y–Z planes of MDA-MB-231 cells cultured on biotinylated collagen in serum-free medium for 48 h and stained for F-actin with phalloidin (blue), for cathepsin B-digested collagen with A7 antibody followed by goat anti-mouse Alexa Fluor 594 (red), and for biotinylated collagen (green). Note the appearance of collagen fragments (yellow arrows) above the substratum associated with the actin in the plane of the cell.

be observed, as was observed for HGFs [4]. Additionally, a high level of staining for lysosomes, which are required for intracellular breakdown of collagen, was evident in the invasive cancer cells and was marked in the HT1080 and MDA-MB-231 cells (Figure 2A). Notably, an increase in lysosomes was observed by electron microscopy in HGFs treated with ConA, which increases MT1-MMP expression and collagen degradation [4]. The invasive cancer cells also stained with the monoclonal antibody A7 (Osteometer, Nordic Bioscience Diagnostics), which recognizes the neoepitope generated by cathepsin B, a result that is consistent with the intracellular degradation of collagen (Figure 2B). However, more definitive evidence of intracellular collagen degradation by cancer cells requires transmission electron microscopy analyses, which are currently under way.

Discussion

In contrast with the rapid, indiscriminate degradation of collagen that is mediated extracellularly by MMPs and which occurs in growing tissues and in pathological destruction, the intracellular degradation of collagen allows for the recognition of the fibres to be degraded. This provides increased selectivity that is important for remodelling collagen in response to functional demands [1] and can provide directed degradation for invasive cancer cells. While MT1-MMP has been shown to be a key enzyme in the fragmentation of collagen fibres that is required for phagocytosis [4], the same enzyme, through its collagenolytic and MMP-2 activation activity, can also function in the extracellular degradation of collagen [14]. Our studies indicate that the more invasive cancer cells expressing MT1-MMP [10,11] may also use the intracellular pathway for directed migration through collagenous matrices. Although the involvement and extent of the utilization of the phagocytic pathway have to be verified,
it is notable that the gelatinase activities of MMP-2 and -9, which can degrade the unfolded collagen fragments generated by collagenase activity, are not required for the phagocytosis of collagen by HGFs, nor for the degradation of collagen by the invasive cancer cells. Despite the apparent ability of the poorly invasive cancer cells to degrade collagen films in an MMP-independent manner, they are unable to effectively penetrate the collagen plugs in Transwell membranes, thereby limiting their invasive capacity.

These studies were supported by grants from Canadian Institutes of Health Research (CIHR), MOP-36333 and MOP-79324, and from the Toronto Ovarian Cancer Research Network. H.L. was supported by a CIHR M.Sc. Studentship Award and K.L.S. by funds raised for ovarian cancer research by the Toronto Fashion Show.

**References**


Received 16 April 2007