Membrane-Type I Matrix Metalloproteinase-Dependent Regulation of Rheumatoid Arthritis Synoviocyte Function

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In rheumatoid arthritis, the coordinated expansion of the synoviocyte mass is coupled with a pathologic angiogenic response that leads to the destructive remodeling of articular as well as surrounding connective tissues. Although rheumatoid synoviocytes express a multiplicity of proteolytic enzymes, the primary effectors of cartilage, ligament, and tendon damage remain undefined. Herein, we demonstrate that human rheumatoid synoviocytes mobilize the membrane-anchored matrix metalloproteinase (MMP), membrane-type I MMP (MT1-MMP), to dissolve and invade type I and type II collagen-rich tissues. Though rheumatoid synoviocytes also express a series of secreted collagenases, these proteinases are ineffective in mediating collagenolytic activity in the presence of physiologic concentrations of plasma- or synovial fluid-derived antiproteinases. Furthermore, MT1-MMP not only directs the tissue-destructive properties of rheumatoid synoviocytes but also controls synoviocyte-initiated angiogenic responses in vivo. Together, these findings identify MT1-MMP as a master regulator of the pathologic extracellular matrix remodeling that characterizes rheumatoid arthritis as well as the coupled angiogenic response that maintains the aggressive phenotype of the advancing pannus. The Journal of Immunology, 2010, 184: 000–000.

In rheumatoid arthritis (RA), the hallmark pathologic remodeling of cartilage, tendons, ligaments, and bone associated with the disease process can be largely ascribed to direct or indirect effects mediated by the RA synoviocyte (1–4). In tandem, RA synoviocyte-initiated angiogenic responses appear to play a critical role in these tissue-destructive events by providing nutrients to the hyperplastic synovium and supporting the ingress of inflammatory cell populations (1–4). Currently, the major mechanisms that confer RA synoviocytes with the ability to destructively remodel the extracellular matrix remain largely undefined, as do the signaling programs that trigger the surrounding vasculature to mount a cooperative angiogenic response.

In terms of the irreversible joint destruction that underlies RA, the critical structural targets are characterized by their high content of interstitial collagens (1–3). Although the cartilaginous matrix is dominated by type II collagen, the extracellular matrix of ligaments, tendons, and bone is largely composed of type I collagen (1–3). Presently, triple-helical collagenases (i.e., those proteinases that are able to hydrolyze collagen by cleaving within triple-helical domains) are limited to members of the matrix metalloproteinase (MMP) and cysteine proteinase families (5–8). Within the MMP family, four secreted collagenases have been identified:

- MMP-1 (the so-called fibroblast collagenase), MMP-8 (collagenase-2), MMP-13 (collagenase-3), and MMP-2 (gelatinase A) (3, 5, 9). Although the type I or type II collagenolytic properties of these secreted proteinases have been most extensively studied, recent work has demonstrated that the membrane-anchored MMPs, MT1-MMP and MT2-MMP, can also exert direct collagenolytic effects (3, 5, 9–12). Further, like the MMP family, the cysteine proteinases also comprise a large group of proteolytic enzymes, but only a single member of this gene family, termed cathepsin K, displays triple-helical collagenolytic properties (7, 8). Significantly, all seven of these collagenases have been identified in the rheumatoid synovium at the mRNA or protein levels, or both (10–19). The precise role, however, that these enzymes play in RA synoviocyte-mediated tissue destruction remains the subject of speculation. Despite the facts that RA synoviocytes are currently believed to mediate the bulk of the tissue-destructive effects associated with this disease state (1–4) and that the isolated cells can be shown to express multiple proteolytic enzymes in vitro (19–27), only a handful of studies have attempted to examine the type I/II collagenolytic properties of isolated synovial fibroblasts or tissues (28–30). In the few cases where the collagenolytic activity of RA synoviocytes has been documented (28–30), the proteolytic systems responsible for collagen degradation have not been identified directly. Herein, we demonstrate that human RA synoviocytes use MT1-MMP as the dominant effector responsible for the degradation and invasion of type I or II collagen-rich structures. Furthermore, synoviocyte MT1-MMP can play an additional role in promoting disease progression by tandemly inducing an angiogenic cascade that serves to couple active matrix degradation with the induction of a neovascularure.

Materials and Methods

Isolation and culture of human RA synoviocytes

Human RA synoviocytes were obtained by collagenase digestion of synovial tissue obtained at arthroplasty or synovectomy from RA or osteoarthritic (OA) joints as described (31). All of the procedures involving specimens obtained from human subjects were performed under protocols approved by the University of Michigan Institutional Review Board. Cells were routinely cultured in Connaught Medical Research Laboratory medium (In-vitrogen, San Diego, CA) supplemented with either 10% heat-inactivated
Combinant tissue inhibitor of metalloproteinases (7.5 μg/ml heat-inactivated human serum were added to the upper chamber. Where free medium was added to each well with or without IL-1

RT-PCR analysis

RA synoviocytes were cultured atop type I collagen in the presence of 1 nM IL-1β (Calbiochem, La Jolla, CA) in media supplemented with 10% heat-inactivated human serum and total RNA isolated using TRIzol reagent (Life Technologies, Rockville, MD). RT-PCR was performed with 1 μg total RNA and 10 μM specific primers (9), using One-Step RT-PCR System Reagent (Life Technologies). The identity of each PCR product was confirmed by sequence analysis (32).

Collagen degradation assays

To determine the type I collagenolytic potential of OA or RA synoviocytes, 12-well plates were coated with a thin layer of acid-extracted, rat tail tendon type I collagen (200 μg per well or 64 μg/cm²) as described previously (9). After gelling, 5 × 10⁵ synoviocytes suspended in 50 μl serum-free medium were seeded into the center of each well and allowed to attach for 8 h (9, 33). After the removal of nonadherent cells by washing, 0.5 ml serum-free medium was added to each well with or without IL-1β (1 nM), plasminogen (20 μg/ml; Calbiochem), the synthetic MMP inhibitor BB-94 (5 μM) interfere with the matrix degradation activities of RA or OA synoviocytes were analyzed in cross sections of the recovered cartilage fragments as described (34, 40).

Ex vivo synoviocyte–cartilage coculture system

Articular cartilage fragments, dissected from the knee joints of New Zealand white rabbits, were cultured with either RA or OA synoviocytes (1 × 10⁶ cells labeled with Vybrant DiO) for 2 h in vitro to allow the cells to adhere to the tissue explants. The synoviocyte–cartilage cocultures were then placed atop the CAM of 11-d-old chick embryos for 4 d. The invasive activities of RA or OA synoviocytes were analyzed in cross sections of the recovered cartilage fragments as described (34, 40).

Histology

Frozen sections of synoviocyte–collagen gel cocultures were incubated with a polyclonal Ab against the denatured fragment of type I collagen (gift of R. Poole, McGill University, Montreal, Canada) (34, 41). Chick type IV collagen was visualized by immunofluorescence using a mouse anti-chicken type IV collagen mAb (40) and Alexa Fluor rabbit anti-goat IgG (Molecular Probes).

Immunofluorescence and light microscopy

Fluorescence images of cell invasion into cartilage fragments or the CAM were captured with a Spot digital camera (Diagnostic Instruments, Sterling Heights, MI) through an upright microscope (Leica Microsystems, Deerfield, IL). Collagen cultures were prepared for light microscopy as described (11, 34), and sections (5- to 7-mm-thick) stained with H&E.

Results

Type I collagenolytic activity of RA synoviocytes

When RA synoviocytes are seeded as a discrete island atop an underlying three-dimensional bed of type I collagen fibrils under serum-free conditions and stimulated with the cytokine, IL-1β, the cells focus degradative activity specifically to their subjacent compartment (i.e., zones of collagenolysis are visualized by staining and degrading remaining collagen fibrils with Coomassie blue so that areas of degradation appear as “cleared” zones; Fig. 1A). Although neither serine nor cysteine proteinase inhibitors (i.e., aprotinin or E-64d, respectively) (9) affect RA synoviocyte-mediated collagenolytic activity, the pan-specific MMP inhibitor BB-94 (9) blocks subjacent degradation completely without affecting cell viability (Fig. 1A and data not shown). Consistent with a dominant role for MMPs in the degradative program displayed by RA synoviocytes, a RT-PCR screen demonstrates that the IL-1β–stimulated cells express the secreted collagenases, MMP-1, MMP-13, and MMP-2, as well as the membrane-anchored collagenases, MT1-MMP and MT2-MMP (Fig. 1B).

Under serum-free conditions, RA synoviocyte-dependent collagenolytic activity is dependent on the ability of the cells to convert MMP zymogens to their catalytically active forms (1–3). In vivo, synoviocytes are bathed in interstitial fluids that contain high concentrations of plasminogen (42). Because RA synoviocytes can process plasminogen to plasmin, which, in turn, effectively converts secreted collagenase zymogens to their active forms (1–3, 43), the collagenolytic potential of IL-1β–stimulated RA synoviocytes was assessed in the presence of exogenous plasminogen. Under serum-free conditions, the addition of plasminogen increases collagenolytic activity >3-fold, with degradative activity extending from the subjacent compartment of the overlying cells to the surrounding field of collagen as assessed either in the Coomassie blue assay or by confocal laser microscopy with fluorescently labeled type I collagen (where synoviocyte–collagen interactions can be monitored at the single-cell level) (Fig. 1C–E). As expected, the enhanced collagenolytic activity supported by exogenous plasminogen is inhibited completely by BB-94 (Fig. 1C–E). When, by contrast, plasmin activity is blocked with aprotinin (i.e., aprotinin does not affect MMP activity directly) (9), RA synoviocyte-mediated collagen degradation collapses back to the subjacent compartment alone (Fig. 1C). Together, these results are consistent with a model wherein synoviocytes degrade surrounding collagen through the
combined attack of 1) membrane-localized MMPs that confine their activity to the subjacent compartment and 2) plasmin-activatable, secreted collagenases that act in a more global context by degrading collagen beyond the boundaries of the pericellular space (9).

The ability of RA synoviocytes to mobilize plasmin-activated, secreted collagenases that act in a more global context by degrading collagen beyond the boundaries of the pericellular space (9).

The ability of RA synoviocytes to mobilize plasmin-activated, secreted collagenases to degrade collagen is dependent upon a set of conditions permissive for productive collisions between catalytically active plasmin and the collagenase zymogens. Although plasmin-dependent collagenase activation by RA synoviocytes is readily observed under serum-free conditions, the efficiency of such interactions is less certain in pathophysiologic states where high concentrations of plasma-borne, endogenous antiproteinases limit the half-life of fluid-phase proteinases (43, 44). Hence, even when the anti-proteinase content of synovial fluid or serum is reduced 10-fold (i.e., by diluting the serum or synovial fluid concentration from 100 to 10%), BB-94-sensitive collagenolytic activity of the RA synoviocyte remains confined to the subjacent compartment.

MT1-MMP confers RA synoviocytes with subjacent collagenolytic activity

At the RA synoviocyte–collagen interface, secreted or membrane-anchored MMPs—working alone or in tandem—could potentially support subjacent collagenolysis (9, 45). As such, each of the MMPs expressed by IL-1β–stimulated RA synoviocytes was...
silenced by MMP-specific siRNAs (confirmed at the mRNA or protein levels; Fig. 2A), and the cells were cultured atop type I collagen gels in the presence of 10% human serum. Under these conditions, silencing MMP-1, MMP-13, or MMP-2 did not inhibit subjacent collagenolysis as assessed by 1) Coomassie blue staining or confocal laser microscopy (Fig. 2B, 2C) or 2) hydroxyproline assay (Fig. 2D). Consistent with the inability of secreted collagensases to mediate subjacent collagenolysis, high concentrations of TIMP-1, an endogenous MMP inhibitor that effectively blocks MMP-1, MMP-13, and MMP-2 activities (9), did not affect degradative activity (Fig. 2D). By contrast, when MT1-MMP, but not MT2-MMP, expression is silenced, type I collagenolytic activity is suppressed completely (Fig. 2B–D). Similarly, the ability of RA synoviocytes to degrade subjacent collagen is blocked in the presence of TIMP-2, a second member of the TIMP family that includes MT1-MMP as a target proteinase (9). The inhibitory effect exerted by the MT1-MMP-directed siRNA is specific with regard to MT1-MMP knockdown because type I collagenolytic activity is rescued fully when the MT1-MMP siRNA-treated RA synoviocytes are transduced with a siRNA-resistant mouse MT1-MMP orthologue (Fig. 2B–D). Importantly, the ability of IL-1β to trigger a robust subjacent collagenolytic response is not a general property of all synoviocyte populations because cells isolated from OA patients, a disease that is seldom characterized by widespread synoviocyte-mediated matrix remodeling (1–3), exhibit <20% of the degradative activity observed in RA synoviocytes (Fig. 2B–D). Consistent with these findings, whereas RA synoviocytes upregulate MT1-MMP levels in response to IL-1β or TNF-α, OA synoviocytes express only barely detectable levels of the protease (Supplemental Fig. 1).

In RA, activated synoviocytes not only degrade type I collagen-rich ligaments and tendons but also display collagen-invasive activity (1–3). To assess the role of MT1-MMP in supporting tissue-invasive activity, RA synoviocytes were next cultured atop three-dimensional gels of type I collagen and stimulated by the addition of 10% human serum. Under these conditions, RA, but not OA, synoviocytes rapidly infiltrate the underlying collagen gel (Fig. 3A, 3B). Coincident with the display of collagen-invasive activity, RA synoviocytes degrade the surrounding collagen fibrils as detected with an Ab specific for MMP-generated neoepitopes that are only exposed following collagenolytic attack (9, 41) (Fig. 3C). In a fashion similar to that observed during subjacent collagenolysis, neither TIMP-1 nor siRNAs directed against MMP-1, MMP-13, or MMP-2 inhibit invasion or the generation of invasion-associated collagen degradation products (Fig. 3). In the presence of TIMP-2, however, or following MT1-MMP—but not MT2-MMP—silencing, both invasive activity and pericellular collagen degradation are ablated (Fig. 3). Though MT1-MMP has been reported to exert effects on cell motility via mechanisms that operate independently of the proteinase’s catalytic activity (9, 46), MT1-MMP–silenced synoviocytes retain their ability to invade noncovalently cross-linked type I collagen gels prepared from pepsin-extracted tissues (Fig. 3B). Because pepsin-extracted gels are devoid of the collagen telopeptide cross-links that dictate matrix structure and rigidity, invasion of these constructs is dependent upon intact motile and mechanical activities alone (9, 46). Likewise, MMPs are not required for RA synoviocyte invasion of non-cross-linked barriers of basement membrane extracts (i.e., Matrigel) (Fig. 3B).

**MT1-MMP directs the RA synoviocyte–cartilage invasion program**

Despite the dominant role played by MT1-MMP during RA synoviocyte degradation or invasion of type I collagen-rich barriers, the cartilaginous matrix that underlies synoviocytes in the joint space is structurally distinct from ligaments or tendons and is dominated by a network of heavily cross-linked type II collagen that is resistant to most forms of proteolytic attack (1–3). To identify the dominant type II collagenase operative in RA synoviocytes, cells were cultured atop fluorescently labeled, type II collagen films following electroporation with a control siRNA or siRNAs directed against MMP-1 and MMP-13, MMP-2, MT1-MMP, or MT2-MMP. As expected, RA synoviocytes effectively degrade the subjacent collagen fibrils (Fig. 4). Silencing the expression of the secreted collagenases did not, however, affect type II collagenolytic activity (Fig. 4). By contrast, all of the type II collagen degradation is blocked when MT1-MMP expression alone is targeted (Fig. 4).

Although RA synoviocytes readily degrade type II collagen, evidence to date indicates that invasion through matrix-dense barriers of articular collagen cannot be induced readily in short-term, in vitro assay systems (2). Alternatively, most studies have relied primarily on in vivo model systems wherein synoviocytes are cocultured with cartilage explants and implanted in immunoincompetent mice for long-term culture (i.e., up to 60 d) (2). In an effort to develop a more tractable in vivo experimental model to assess dynamic synoviocyte–cartilage interactions, human RA synoviocytes were cultured with live articular cartilage and implanted atop the CAM of the 11-d-old developing chick, a highly vascularized organ that readily supports the functional and structural integrity of explanted tissues (Fig. 5A) (47). In the immuno-incompetent setting of the chick embryo, RA synoviocytes (labeled with fluorescent tracking dye) rapidly invade the cartilage matrix during a 4-d culture period (Fig. 5B, 5C). By contrast, OA synoviocytes, a cell population that does not readily express cartilage-invasive behavior (2), are unable to mount an infiltrative response in the chick model (Fig. 5B, 5C). To next determine the relative roles of the major secreted versus membrane-anchored MMPs in supporting cartilage-invasive activity, RA synoviocyte expression of MMP-1, MMP-13, MMP-2, or MT1-MMP was silenced, the cells were cultured atop the surfaces of the cartilage explants, and the cell–tissue conglomerate was incubated atop the chick CAM. Under these conditions, only MT1-MMP siRNA inhibits cartilage invasion via a process that is reversed completely following transduction with the mouse MT1-MMP orthologue (Fig. 5B, 5C).

**MT1-MMP–dependent induction of RA synoviocyte-mediated angiogenesis**

Angiogenesis plays a key role in supporting the destructive matrix remodeling program associated with RA, but the identity of the key upstream mediators remains largely uncharacterized (4). Recent studies have demonstrated that RA synovial tissue can induce a potent angiogenic response when transplanted atop the chick CAM (48). As such, an inoculum of fluorescently labeled RA synoviocytes was inoculated directly atop the chorioallantoic surface for a 4-d culture period. Under these conditions, RA synoviocytes infiltrate the chick tissues and coincidentally initiate a robust angiogenic response (Fig. 6A–C). When, however, MT1-MMP is silenced, not only is RA synoviocyte invasion into the chick stromal tissues inhibited completely, but the associated angiogenic response is blocked as well (Fig. 6A–C). The inhibitory effects of the MT1-MMP siRNA on invasion and angiogenesis in the chick system are reversed following MT1-MMP rescue with the mouse orthologue (Fig. 6A–C).

To rule out the possibility that angiogenic responses are engaged only as an indirect consequence of chick tissues having been infiltrated by a foreign cell population, the CAM was alternatively overlaid with a preformed, three-dimensional sheet of type I collagen.
FIGURE 2. Relative roles of secreted and membrane-anchored MMPs in synoviocyte-mediated type I collagenolytic activity. A, RT-PCR analysis of MMP expression of IL-1β–stimulated RA synoviocytes seeded atop type I collagen gels 48 h postelectroporation with control, MMP-1–, MMP-13–, MMP-2–, MT1-MMP–, or MT2-MMP–specific siRNA constructs (lower band of MT1-MMP doublet is non-specific). Representative Western blots and zymograms demonstrating effective silencing of MMP-1, MMP-13, MT1-MMP, and MMP-2 expression in RA synoviocytes are shown (middle and right panels). β-Actin is used as a loading control. B and C, Type I collagen degradation by siRNA-electroporated RA or OA synoviocytes cultured atop type I collagen gels following a 5-d culture period in the presence of IL-1β and 10% human serum as monitored by either Coomassie staining (B, scale bar, 20 mm) or by confocal laser microscopy of rhodamine-labeled collagen (red), phalloidin, and DAPI labeled cells (green and blue) (C, original magnification ×40; scale bar, 100 μm). The collagenolytic activity of MT1-MMP siRNA-electroporated RA synoviocytes is rescued following expression of mouse MT1-MMP (mMT1 rescue). Results are representative of four or more performed. D, Hydroxyproline release from RA or OA synoviocytes cultured atop type I collagen gels for 5 d in the presence of 10% human serum and IL-1β (10 nM) was monitored following siRNA electroporation. Alternatively, RA or OA synoviocytes were cultured in 10% human serum with L-1β in the absence or presence of BB-94, TIMP-1, or TIMP-2. Results are expressed as the mean ± SEM of four experiments.
collagen alone or a collagen sheet upon whose apical face a confluent layer of RA synoviocytes was established. In the presence of a naked collagen overlay, only chick heterophils are found infiltrating the matrix explant (Fig. 6D). By contrast, when the surface of the type I collagen gel was overlaid with RA, but not OA, synoviocytes, the applied collagen matrix undergoes marked neovascularization (Fig. 6D, 6E). Although silencing neither MMP-1, MMP-13, nor MMP-2 expression in the RA synoviocytes affects the angiogenic response, MT1-MMP–silenced RA synoviocytes are incapable of initiating neovessel formation unless MT1-MMP expression is rescued by the mouse MT1-MMP orthologue (Fig. 6D, 6E). Hence, RA synoviocytes are not only reliant on MT1-MMP for initiating tissue-invasive programs but also for triggering angiogenic responses in an in vivo setting.

Discussion

Human RA synoviocytes invade and degrade the collagen-rich structures associated with joint tissues including tendons, ligaments, bone, and cartilage (1–3). Although the human genome encodes >500 proteases, only a small subset of these gene products—confined largely to the MMP and cysteine proteinase families—can degrade intact type I or II collagen molecules within their triple-helical domains (5–11). RA synoviocytes have been reported to express multiple collagenases (1–3, 12–19), but the dominant effectors capable of supporting either collagen-invasive or collagen-destructive phenotypes under pathophysiologically relevant conditions have remained the subject of controversy (3). Herein, we have demonstrated that MT1-MMP alone confers RA synoviocytes with the ability to degrade or invade cross-linked collagen networks under conditions in which secreted collagenase activity is quenched effectively by serum- or synovial fluid-derived antiproteinases.

Prior to the discovery of the MT-MMPs, early efforts to identify the collagenolytic activities responsible for the destructive tissue remodeling that characterizes RA focused largely on synoviocyte-derived MMP-1 or MMP-13 (2, 3, 28–30). Indeed, more than 30 y ago, Werb et al. (30) and colleagues first demonstrated that human

FIGURE 3. MT1-MMP mediates RA synoviocyte collagen-invasive activity. A–C, OA or RA synoviocytes were cultured atop three-dimensional gels of type I collagen (2.2 mg/ml) in 10% human serum for 4 d in the absence or presence of TIMP-1 or TIMP-2, or following siRNA electroporation. H&E-stained cross sections show that OA synoviocytes are confined to the upper surface of the collagen gel (marked by arrow to the left of the upper panels), whereas RA synoviocytes invade into the underlying matrix (marked by arrows in the second panel, top row). The double-headed arrow marks the thickness of the collagen gel (first panel, top row; scale bar, 100 μm). Original magnification ×40. In B, the number of invasive synoviocytes that have infiltrated either cross-linked, acid-extracted type I collagen, or pepsin-extracted type I collagen (i.e., PureCol) or Matrigel was determined in 10 randomly selected fields in a single representative experiment of three performed. Results are expressed as the mean ± SEM. In C, immunofluorescent staining of type I collagen degradation products in cross sections reveals zones of denatured collagen Alexa Fluor 488 conjugated Ab, (green fluorescent signal, white arrows) surrounding the invading synoviocytes (propidium iodide labeled) in the absence or presence of TIMP-1 but not in the presence of TIMP-2 or following MT1-MMP silencing (scale bar, 100 μm).

FIGURE 4. Type II collagenolytic activity of RA synoviocytes. Collagenolytic activity of RA synoviocytes electroporated previously with a control siRNA or siRNAs directed against MMP-1, MMP-13, MMP-1 and MMP-13, MMP-2, MT1-MMP, or MT2-MMP and cultured atop type II collagen films in the presence of IL-1β was monitored after a 5-d culture period by confocal laser microscopy of Alexa Fluor 488-labeled type II collagen. At the end of the culture period, synoviocytes were stained with Alexa Fluor 568 (red) phalloidin and DAPI (blue) prior to visualization. Results are representative of three performed (original magnification ×40; scale bar, 100 μm).
RA synoviocytes cultured under serum-free conditions and supplemented with plasminogen were able to mount potent collagenolytic activity. Although the relative roles of MMP-1 versus MMP-13 were not assessed in this model (MMP-13 had not yet been identified), the issue of how the collagenase zymogen(s) might undergo extracellular activation in the presence of a relative excess of plasma antiproteinases was considered (30). In their study, the authors postulated that synoviocytes “penetrate” the antiproteinase shield by generating quantities of proteinases that exceed the local concentration of proteinase inhibitors (e.g., plasma proteinase inhibitors directed against plasmin or MMP-1, α2-antiplasmin, and TIMP-1, respectively, bind and inhibit target proteinases at a 1:1 molar ratio) (30, 43). Later work demonstrated that the antiproteinase shield could be further diminished by leukocyte-derived oxidants, but only a limited number of in vivo situations have been identified wherein the local proteinase concentration exceeds that of the antiproteinases (43). Indeed, in our studies, even dilute concentrations of serum or synovial fluid effectively quenched the activity of the secreted collagenases. The inability of RA synoviocytes to mobilize secreted collagenases to effect a collagenolytic phenotype in the presence of circulating antiproteinases is not meant to suggest that these matrix-degrading proteinases are barred from supporting destructive tissue remodeling under all in vivo conditions. In the RA joint, the combined presence of neutrophils, macrophages, lymphocytes, and synoviocytes could conceivably generate an oxidative and proteolytic burden that together overwhelms the local antiproteinase shield (1–3, 43). Nevertheless, RA synoviocytes are frequently found as the exclusive cell population at the tissue-invasive front in vivo (1–3, 49). Under these conditions, we posit that only a limited repertoire of collagenolytic options is available to the RA synoviocyte. Because MT1-MMP 1) degrades both type I and type II collagen, 2) undergoes processing from its zymogen to catalytically active form within the trans-Golgi network (an intracellular compartment inaccessible to extracellular antiproteinases), and 3) is tethered to the plasma membrane at the cell–matrix interface (a subjacent compartment in which circulating antiproteinases have limited access) (8–11, 50), the metalloenzyme is ideally suited to serve as the dominant collagenase operative within the pericellular milieu of the RA synoviocyte. Indeed, increasing the serum or synovial fluid concentration to 50% fails to blunt MT1-MMP–dependent collagenolytic activity (data not shown), and the
proteolytic system is equally effective when apprised within antiproteinase-rich chick tissues in an in vivo setting. Though MT2-MMP is also able to act as a pericellular collagenase (10, 11) and could be detected by RT-PCR in RA synoviocytes, we were unable to link this proteinase to the collagenolytic phenotype. Because we have not yet identified MT2-MMP–specific Abs, the possibility that the MT2-MMP protein is expressed at only low levels relative to MT1-MMP, or that the enzyme fails to localize to the cell–matrix interface, requires further study.

Independent of its collagen-degradative potential, we demonstrate that MT1-MMP also confers RA synoviocytes with the ability to invade type I and type II collagen-rich tissues. In vitro, covalently cross-linked three-dimensional networks of purified type I collagen can be assembled from acid-extracted rat tail tendon (46). With native type I collagen gels as a model tissue, MT1-MMP alone supports invasive activity, a finding consistent with recent studies from our laboratory demonstrating that secreted collagenases—though effective collagenolysins—are unable to drive invasion programs (9, 46). To date, only membrane-tethered collagenolytic activity has been shown to effectively coordinate pericellular proteolysis with the dynamic adhesive interactions required for three-dimensional, tissue-invasive programs (9, 46, 50). Importantly, MT1-MMP–silenced RA synoviocytes display no functional defects in their ability to infiltrate noncovalently cross-linked tissue constructs assembled from pepsin-extracted collagen or Matrigel (BD Biosciences, Bedford, MA). These results serve to reinforce the conclusion that MT1-MMP does not play a required role in controlling cell motility or three-dimensional migration.

**FIGURE 6. MT1-MMP–initiated angiogenic activity.** A, RA synoviocytes electroporated with a control or MT1-MMP siRNA were labeled with DiO (green) and seeded atop the CAM of 11-d-old chick embryos and cultured for 4 d. Fluorescent micrographs of CAM cross sections demonstrate the ability of RA synoviocytes to penetrate the CAM surface into the underlying interstitium. Nuclei of all of the cells were stained with DAPI (blue). The dashed white line marks the CAM surface. RA synoviocyte invasion was associated with neovessel formation as observed following staining with an anti-chick type IV collagen mAb that reacts with vascular basement membranes (red, white arrows). MT1-MMP–silenced RA synoviocytes fail to initiate an angiogenic response unless engineered to re-express the protease. Results are representative of four performed (original magnification ×40, scale bar, 100 μm). B and C, Invasion (B) and angiogenesis (C) scores were determined by quantifying green and red fluorescence signals, respectively, within the CAM stroma (mean ± SEM; n = 4). D, Type I collagen gels were assembled in 10-mm Transwell chambers and placed atop the chick CAM for a 4-d culture period during which chick cells infiltrated the tissue construct (left panel, top row). By contrast, when RA synoviocytes were cultured atop the collagen gel, a robust angiogenic response was initiated (arrows in right panel, top row). After MT1-MMP silencing, angiogenesis was ablated in a fashion that is rescued following expression of mouse MT1-MMP (H&E stain of collagen cross section, original magnification ×40, scale bar, 50 μm). Results are representative of three experiments performed. E, Neovessel number was determined in 10 randomly selected fields in a single representative experiment of three performed.
when confronting tissue barriers whose fibrillar components can be remodeled by mechanical forces alone (46). Although we predict that MT1-MMP likewise mediates cartilage-destructive activity directly by degrading type II collagen, three-dimensional type II collagen gels cannot be reconstituted in vitro (i.e., type II collagen can only be extracted from intact cartilage via limited proteolysis of the type II collagen telopeptides). Despite this limitation, only MT1-MMP confers RA synoviocytes with the ability to proteolyze triple-helical type II collagen fibrils, whereas our synoviocyte–cartilage xenograft model demonstrates clearly that MT1-MMP is a required effector of cartilage invasion programs. In contrast with long-held paradigms in the current literature that redundant proteolytic mechanisms can be mobilized to compensate for the loss of MT1-MMP activity, we are unable to identify alternate mechanisms that support type II collagen degradation or cartilage invasion. Interestingly, an absolute requirement for MT1-MMP in RA synoviocyte-mediated cartilage remodeling is consistent with the fact that MT1-MMP–null mice display major defects in cartilage resorption during development and rely inappropriately on recruited osteoclasts to remodel residual cartilage fields by cathepsin K–dependent activity (51). The possibility remains, however, that RA synoviocytes use MT1-MMP to attack other cartilage components important for structural integrity, including type IX and XI collagen as well as the proteoglycans (e.g., aggrecan) that control cartilage hydration and resiliency (3, 52).

A dominant in vivo role for MT1-MMP in RA assumes, in part, expression of the proteinase at sites of active invasion within affected tissues. While our work was in preparation, Miller et al. (53) reported that MT1-MMP is expressed in human RA pannus tissue, particularly within synoviocyte populations localized to the pannus–cartilage junction. Corroborating their findings, we also found that the tissue-invasive properties of isolated RA synoviocytes could be blocked by TIMP-2 but not TIMP-1 (53). Although the specific roles of secreted collagenases and MT-MMPs were not evaluated directly in their work, RA synoviocyte invasion was inhibited when cells were infected with an adenoviral vector encoding a MT1-MMP dominant-negative fragment previously reported to interfere with the catalytic activity of the wild-type enzyme (53). At this juncture, however, the utility of this indirect approach for implicating MT1-MMP can only be embraced cautiously because given that others have reported that the MT1-MMP fragment enhances, rather than inhibits, MT1-MMP proteolytic activity (54). Further, the possibility that the MT1-MMP dominant-negative construct exerts more complex effects on cell function by sequestering signaling molecules that normally bind to the MT1-MMP cystolic tail has not yet been evaluated (55). In a similar fashion, an earlier report documented the ability of a MT1-MMP antisense construct to partially inhibit RA synoviocyte cartilage invasion in a SCID mouse-xenograft model (56). In this case, MT1-MMP activity was not inhibited completely, and the specificity of the antisense construct for MT1-MMP alone was not established. Hence, critical proofs implicating MT1-MMP directly in type II collagen degradation or invasion have, until now, remained suggestive but not definitive.

In RA, the synovium expands to create the pannus, a highly vascularized granulation tissue that is composed largely of synoviocytes, macrophages, and T cells (1, 4). Because the rheumatoid pannus actively invades and destroys the underlying cartilage as well as subchondral bone, early studies likened this invasive amalgam to a tumor-like tissue (1, 4). Indeed, in a manner akin to their neoplastic counterpart, angiogenesis likewise plays a critical role in both promoting and supporting the tissue-destructive behavior of rheumatoid pannus tissue (4). In this regard, we extend a recent report demonstrating the ability of RA, but not OA, tissue explants to initiate a potent angiogenic response in the chick CAM by demonstrating that RA synoviocytes can directly induce these effects in a cell autonomous fashion (48). Given the ability of RA synoviocytes to express a wide range of proangiogenic factors (including cytokines, growth factors, and chemokines) (4), we were surprised to find that MT1-MMP–silenced synoviocytes lose their ability to initiate neovascularization, raising the possibility that MT1-MMP also acts as a novel proangiogenic factor—a conclusion similar to that reached recently in studies of MT1-MMP function in cancer cell populations undergoing an epithelial–mesenchymal cell transition (40). Although the precise mechanism(s) underlying MT1-MMP–induced angiogenic responses remain to be characterized, MT1-MMP has been reported to modulate vascular endothelial growth factor expression, regulate basic fibroblast growth factor activity, and mediate the membrane shedding of proangiogenic members of the semaphorin family (57–61). Interestingly, recent studies have also demonstrated the ability of collagen fragments to induce vascular endothelial growth factor expression by binding to the CXCR class of chemokine receptors (62). This latter finding raises the intriguing possibility that MT1-MMP–dependent collagenolysis initiates a cascade of proteolytic events whereby collagen degradation not only creates conditions permissive for synoviocyte invasion but also initiates an angiogenic response (62, 63). Regardless of the precise mechanism(s) involved, our studies indicate that a single, RA synoviocyte-derived proteinase (i.e., MT1-MMP) can mediate tissue-destructive remodeling, invasion, and angiogenesis in a tandem fashion.

Despite the potential importance of MT1-MMP in controlling RA synoviocyte behavior, little is known with regard to the control of its expression or activity. Within the diseased joint space, MT1-MMP is not only regulated at the transcriptional level but also by complex processes that control its activation, exocytosis to the cell surface, and intracellular recycling (50). Given the multiplicity of cytokines, growth factors, matrix degradation products, and lipid mediators found within the proinflammatory environment that characterizes RA (1–4), it will likely prove difficult to identify a dominant MT1-MMP regulator (50). Indeed less “classical” inflammatory mediators such as hypoxia, HIF-2β, Wnt family members, or epigenetic changes in DNA methylation may well serve as dominant players in the control of MT1-MMP expression or activity in RA (64–69). Nevertheless, given the expression of MT1-MMP within RA synoviocytes at sites of tissue damage in vivo, its pathophysiologically relevant functional properties, and the recent development of MT1-MMP–specific inhibitors (70), the proteinase could serve as an important target for therapeutic intervention.

Disclosures

The authors have no financial conflicts of interest.

References

MT1-MMP–DEPENDENT REGULATION OF SYNOVIOCYTE FUNCTION


Supplemental Figure 1.

Human RA or OA synoviocytes were cultured atop a bed of type I collagen fibrils in the absence of presence of IL-1β (1 nM) or TNF-α (5 nM) for 48 hrs. Western blot analysis of MT1-MMP and α-actin expression were determined in lysates containing 20 μg total protein. MT1-MMP was detected with an anti-MT1-MMP monoclonal antibody (LEM-2/15.8; Millipore).
Supplementary Figure 1

MT1-MMP
α-Actin

RA +IL-1β +TNF-α
OA +IL-1β +TNF-α

kDa
82
64
49
37
26