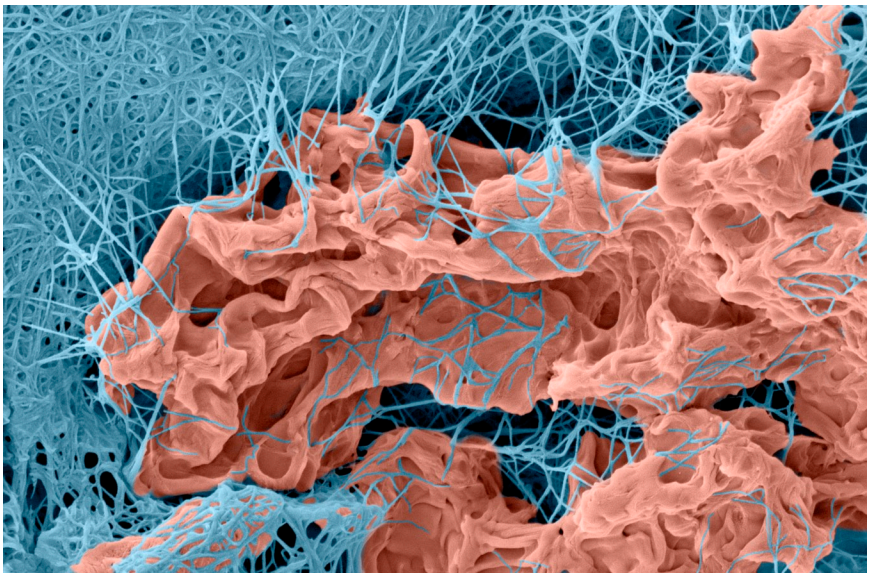
Protease-dependent versus -independent cancer cell invasion programs: three-dimensional amoeboid movement revisited

Farideh Sabeh, Ryoko Shimizu-Hirota, and Stephen J. Weiss

Division of Molecular Medicine and Genetics, Department of Internal Medicine, Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109

Tissue invasion during metastasis requires cancer cells to negotiate a stromal environment dominated by cross-linked networks of type I collagen. Although cancer cells are known to use proteinases to sever collagen networks and thus ease their passage through these barriers, migration across extracellular matrices has also been reported to occur by protease-independent mechanisms, whereby cells squeeze through collagen-lined pores by adopting an ameboid phenotype. We investigate these alternate models of motility here and demonstrate that cancer cells have an absolute requirement for the membrane-anchored metalloproteinase MT1-MMP for invasion, and that protease-independent mechanisms of cell migration are only plausible when the collagen network is devoid of the covalent cross-links that characterize normal tissues.



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3D cancer cell invasion. Multicellular spheroids of HT-1080 fibrosarcoma cells were embedded within 3D gels of native type I collagen for 3 d. Gels were fixed in 2% glutaraldehyde/1.5% paraformaldehyde in 0.1 M sodium cacodylate buffer, freeze-fractured, and processed for SEM. Images of infiltrating cells and the surrounding ECM were digitally imaged using XStream imaging software.

Barriers to cell migration: prevailing theories

After neoplastic transformation, cancer cells infiltrate local tissues and initiate metastatic programs by trafficking through a stromal extracellular matrix (ECM) dominated by cross-linked networks of type I collagen, the major extracellular protein found in mammals (Brown et al., 2003; Sabeh et al., 2004; Demou et al., 2005; Oldberg et al., 2007; Magzoub et al., 2008). To negotiate this structural barrier, cancer cells have been proposed to use either protease-dependent or protease-independent invasion schemes (Wolf et al., 2003a,b, 2007; Sabeh et al., 2004; Wilkinson et al., 2005; Carragher et al., 2006; Wyckoff et al., 2006; Gaggioli et al., 2007; Croft and Olson, 2008; Gadea et al., 2008; Li et al., 2008; Pinner and Sahai, 2008; Sanz-Moreno et al., 2008; Packard et al., 2009). Protease-dependent invasion programs rely on matrix metalloproteinase (MMP) family members to cleave impeding collagen fibrils (Sabeh et al., 2004; Fisher et al., 2006; Hotary et al., 2006; Itoh and Seiki, 2006; Li et al., 2008; Packard et al., 2009). Alternatively, negotiation of collagenous barriers has been reported to proceed in a protease-independent fashion, whereby cancer cells use actomyosin-based mechanical forces to physically displace matrix fibrils while coordinately adopting an amoeboid-like cell shape similar to that observed in myeloid cell populations (Friedl and Wolf, 2003; Wolf et al., 2003a,b, 2007; Wilkinson et al., 2005; Carragher et al., 2006; Wyckoff et al., 2006; Gadea et al., 2008; Pinner and Sahai, 2008; Sanz-Moreno et al., 2008). Indeed, in recent clinical trials, the failure of MMP inhibitors to prevent cancer

Correspondence to Stephen J. Weiss: sjweiss@umich.edu

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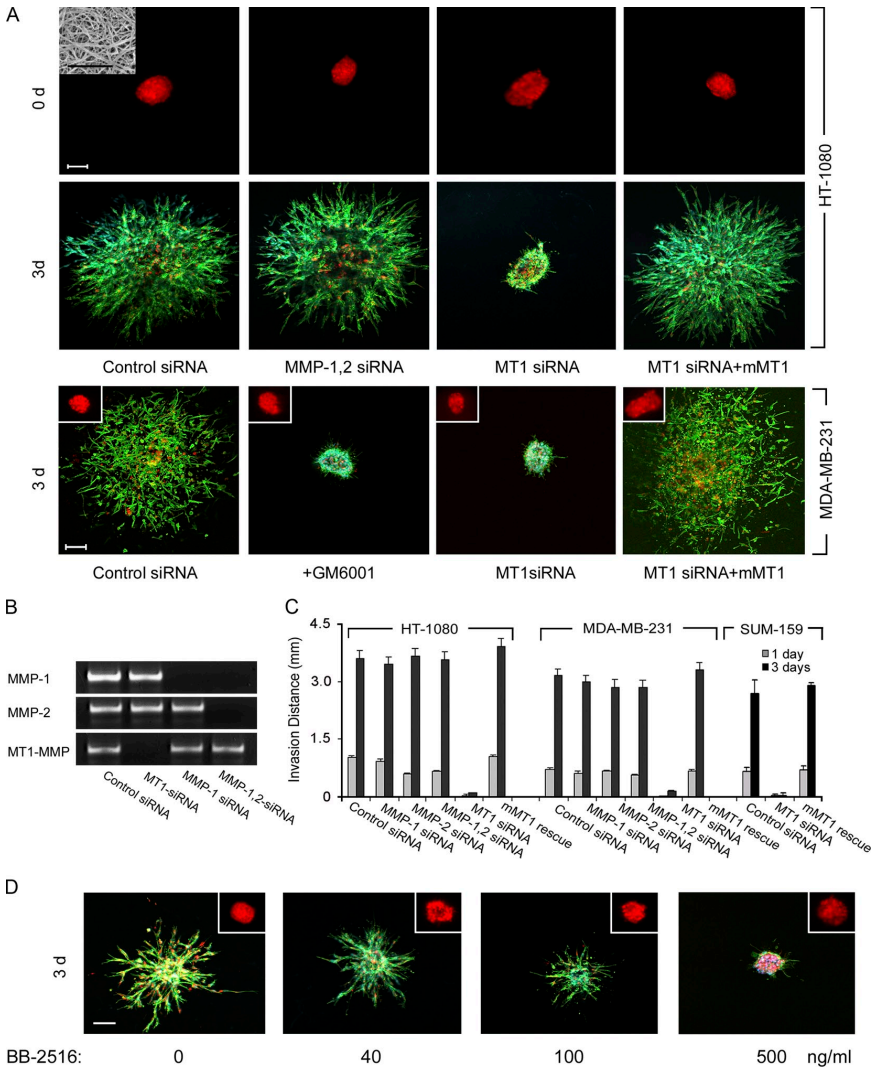


Figure 1. **Regulation of cancer cell–invasive phenotype in 3D type I collagen by MT1-MMP.** (A) Di-I–labeled multicellular spheroids (150–200 µm in diameter) of HT-1080 cells or MDA-MB-231 cells were prepared in hanging droplets (Kelm et al., 2003) after electroporation with a control siRNA, MMP-1 and MMP-2 siRNAs, MT1 siRNA (50–100 nM; siRNA sequences can be found in Li et al. [2008] and Sabeh et al. [2004]), or cotransfected with MT1 siRNA and either mouse (m)MT1-MMP or a control expression vector (0.5 µg each in PCR3.1 Uni; gift of M. Seiki, University of Tokyo, Tokyo, Japan; Sabeh et al., 2004; Li et al., 2008), and embedded in 3D type I collagen gels (prepared from acid extracts of rat tail tendon as described previously [Sabeh et al., 2004] and visualized by SEM in the left-hand panel, top corner inset; bar = 5 µm) at a final concentration of 2.2 mg/ml in the absence or presence of 10 µM GM6001. Invasion was monitored by fluorescence microscopy at 0 d (top row of panels) and by confocal microscopy at 3 d after staining with Alexa-488 phalloidin and DAPI (bottom row of panels; Di-I, red; Alexa-488 phalloidin, green; DAPI, blue). For MDA-MB-231 cells (bottom row of panels), spheroid at 0 d are shown in the insets. Bar = 100 µm. (B) MT1-MMP, MMP-1, and MMP-2 siRNAs inhibited expression of the targeted MMPs in HT-1080 cells, but had no effect on the expression of nontargeted MMPs as shown by RT-PCR. (C) Invasion distance (quantified as described previously; [Hotary et al., 2000; Sabeh et al., 2004]) from the inoculation site into the surrounding matrix was monitored at 1 d and 3 d for HT-1080, MDA-MB-231, or SUM-159 cells. In the presence of a protease inhibitor cocktail directed against serine, aspartyl, cysteinyl, and matrix metallo-proteinases (Wolf et al., 2003a), HT-1080 invasion was inhibited by 99 ± 0%. Results are expressed as mean ± SEM (n = 4). (D) HT-1080 cell spheroids were embedded in type I collagen gels in the absence or presence of the indicated doses of BB-2516 for 3 d. Insets show the spheroid at 0 d after Di-I labeling and the migration of the cells after 3 d is shown in the bottom row of companion panels. Bar = 100 µm. The percentage of inhibition of invasion at 40, 100, and 500 ng/ml (1.5 µM) BB-2516 was 59 ± 4%, 86 ± 2%, and 99 ± 0%, respectively.

progression suggests that protease-independent mechanisms of invasion may be physiologically relevant in vivo (Friedl and Wolf, 2003; Wilkinson et al., 2005; Sahai et al., 2007; Wolf et al., 2007). However, descriptions of proteinase-independent, amoeboid-like cancer cell behavior are largely drawn from in vitro assays using model, three-dimensional (3D) ECM constructs that may not recapitulate the key structural characteristics displayed by native type I collagen networks in vitro or in vivo (Sabeh et al., 2004; Demou et al., 2005; Hotary et al., 2006; Packard et al., 2009). As such, the relative importance of protease-dependent and -independent invasion modalities mobilized during cancer cell trafficking through interstitial barriers remains a subject of considerable debate.

MT1-MMP, synthetic MMP inhibitors, and the 3D cancer cell invasion program

To recreate an in vivo–like environment for migrating cancer cells, multicellular spheroids of HT-1080 fibrosarcoma cells were embedded within 3D gels of native type I collagen (Hotary et al., 2003; Sabeh et al., 2004; Li et al., 2008). HT-1080 cells subsequently activate a tissue-invasive program and infiltrate the surrounding ECM in a "starburst" pattern (Fig. 1 A). Migrating HT-1080 cells express at least three distinct type I collagenolytic systems under these conditions: the secreted metalloenzymes, MMP-1 and MMP-2, as well as the membrane-anchored proteinase, MT1-MMP (Fig. 1 B) (Sabeh et al., 2004; Li et al., 2008). After siRNA-dependent silencing of both MMP-1 and MMP-2 in combination, HT-1080 spheroids retain full invasive activity (Fig. 1, A–C). In contrast, MT1-MMP silencing blocks virtually all invasive activity over a 3-d culture period (Fig. 1 A). Invasive activity is, however, rescued when MT1-MMP–silenced cells are electroporated with an expression vector for mouse MT1-MMP, an orthologue that escapes targeting by the human-specific MT1-MMP siRNA (Fig. 1, A and C). Similar results are obtained

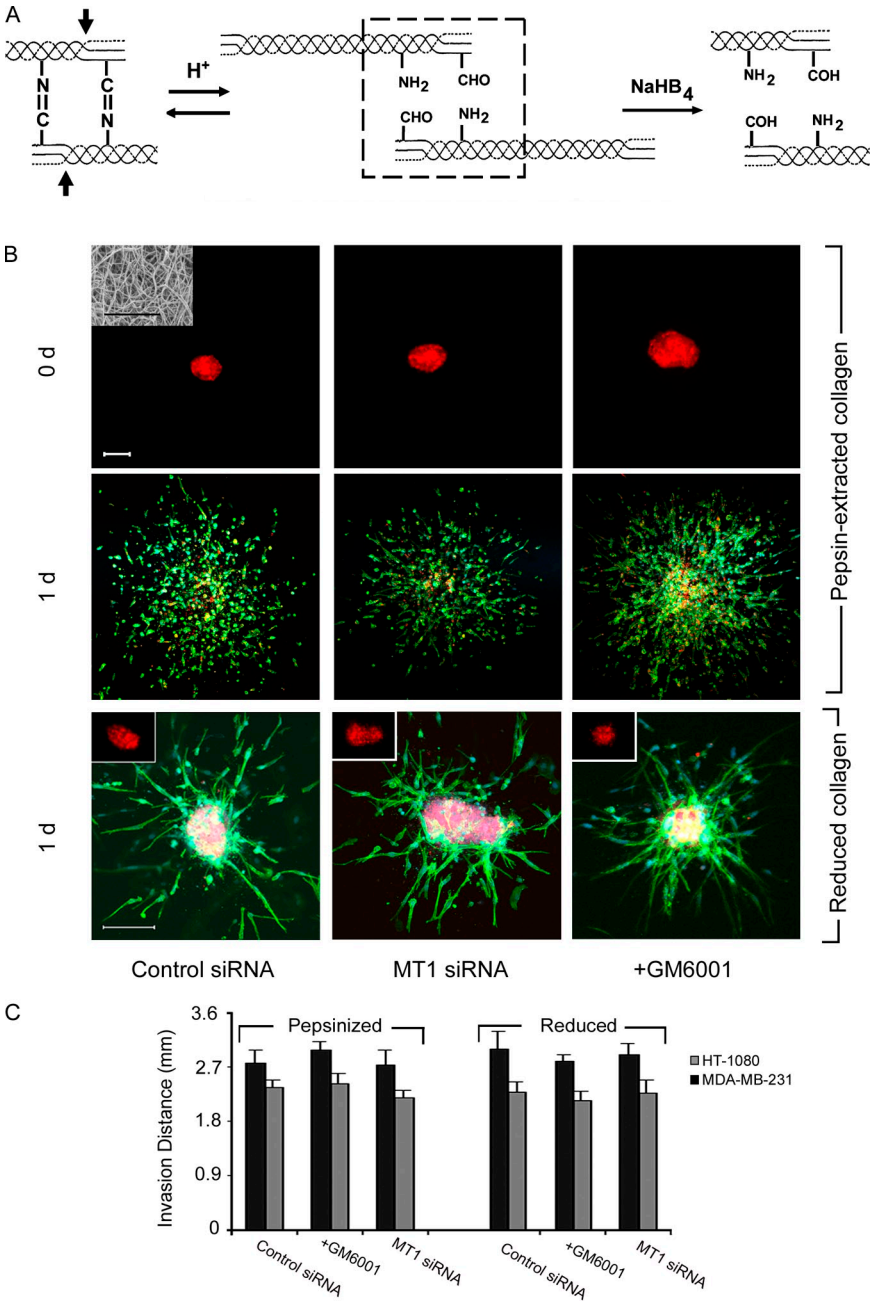


Figure 2. **MT1-MMP–independent tumor cell invasion through telopeptide-excised or cross-link–deficient type I collagen gels.** (A) Schematic of type I collagen intermolecular cross-links. In vivo, lysyl oxidase generates aldehyde moieties within the N- and C-terminal telopeptide domains of type I collagen, which are arrayed across from ε-amino groups (boxed region) that spontaneously condense to conform Schiff base, aldimine cross-links in vivo. N- and C-terminal telopeptides are removed during pepsin extraction (arrows). Under acidic extraction conditions, Schiff base formation is reversed to generate the starting aldehyde and amine groups. After treatment with sodium borohydride, aldehydes are converted to alcohols that no longer support cross-linked formation. (B) Di-I-labeled multicellular spheroids of HT-1080 cells, electroporated with control or MT1 siRNA, were embedded in 3D gels prepared from either pepsin-extracted bovine dermis (Vitrogen; 2.2 mg/ml) or pepsin-extracted rat tail tendon and monitored by fluorescence microscopy at 0 d (top row of panels) and after 1 d (second row of panels; Di-I, red; Alexa-488 phalloidin, green; DAPI, blue). SEM of pepsin-extracted collagen gels is shown in the inset of a 0-d spheroid electroporated with control siRNA (left-hand panel in the first row; bar = 5 µm). GM6001 (10 µM) was added to the cultures at 0 d in the right-hand column of panels. Bar, 100 µm. In the bottom row of panels, Di-I-labeled HT-1080 spheroids were embedded in borohydride-reduced, rat tail type I collagen gels (2.2 mg/ml) at 0 d (insets) and invasion monitored 1 d later after electroporation with a control siRNA, MT1-MMP siRNA or after culture in the presence of GM6001. Collagen aldehyde content before and after borohydride reduction was determined spectrophotometrically (Williams et al., 1978; Gelman et al., 1979). After the 1-d culture period, cells were stained with Alexa-488 phalloidin and DAPI as described above. Bar, 100 µm. (C) HT-1080 or MDA-MB-231 spheroids were cultured for 1 d in 3D pepsinized or reduced type I collagen and invasion distance into the surrounding matrix quantified. HT-1080 invasion in the presence of a protease inhibitor cocktail (Wolf et al., 2003a) in pepsinized or reduced collagen gels was inhibited by 0 ± 0% and 8 ± 1%, respectively. Results are expressed as mean ± SEM ($n = 4$). Acid-extracted, pepsin-extracted, and reduced collagen fibrils are equally trypsin resistant (not depicted).

with MT1-MMP–silenced MDA-MB-231 breast cancer cells or the breast cancer stem cell–enriched SUM-159 line (Fig. 1, A and C) (Korkaya et al., 2008).

The impact of silencing MT1-MMP alone on cancer cell–invasive activity stands in contrast with the failure of peptidomimetic MMP inhibitors (e.g., BB-2516/marimastat) to exert beneficial effects in previously reported clinical cancer trails (Overall and Kleifeld, 2006). Importantly, however, the peak plasma concentrations recorded in most patients (i.e., ∼10–40 ng/ml) (Sparano et al., 2004) falls below the concentration required to completely block MT1-MMP–dependent invasive activity in 3D assays (i.e., ∼500 ng/ml; Fig. 1 D). Thus, the ineffectiveness of MMP inhibitors to exert palliative effects in cancer patients may reflect a requirement for drug concentrations in excess of those achievable in the clinical setting, and does not necessarily support the existence of protease-independent invasive activity in vivo.

Type I collagen cross-links dictate the rules of invasion

The inability of MT1-MMP–silenced HT-1080 or MDA-MB-231 cells to adopt an invasive, amoeboid phenotype-type when embedded in 3D type I collagen gels contradicts recent reports using identical cell lines (Wolf et al., 2003a,b, 2007; Demou et al., 2005; Carragher et al., 2006). However, matrices used in these studies were reconstituted from pepsin-extracted type I collagen (e.g., Vitrogen), a proteolytic process that removes the nonhelical telopeptides situated at the N- and C-terminal ends of native collagen molecules (Fig. 2 A) (Wolf et al., 2003a,b, 2007; Demou et al., 2005; Carragher et al., 2006; Gadea et al., 2008; Sanz-Moreno et al., 2008).

These collagen telopeptides play important roles in fibrillogenesis and contain critical lysine residues which, after lysyl oxidase-dependent oxidation in vivo, support the intermolecular covalent cross-links necessary for stabilizing gel architecture (Gelman et al., 1979; Brennan and Davison, 1980; Eyre et al., 1984; Woodley et al., 1991; Christiansen et al., 2000; Sato et al., 2000). It is therefore possible that the ability of cancer cells to display proteinase-independent, amoeboid activity may be affected by the structural integrity of the reconstituted collagen gel used for in vitro assay.

Following gel formation under conditions identical to those used for full-length, native type I collagen, pepsin-extracted collagen form fibrillar networks indistinguishable from those obtained with telopeptide-intact collagen as assessed by SEM (Fig. 2 B). However, whereas control collagen gels are insoluble in high salt buffers, collagen matrices prepared from pepsin-extracted material rapidly solubilize, a physicochemical behavior characteristic of cross-linked versus noncross-linked collagen gels, respectively (Gelman et al., 1979). HT-1080 spheroids embedded in pepsin-extracted collagen gels infiltrate the matrix in a single-cell fashion with the invasive front displaying a mix of both spheroid- and elongated-shaped cells (Fig. 2 B). Within 24 h, migrating HT-1080 cells invade the pepsin-extracted gels to distances that require a 72-h incubation period in full-length type I collagen matrices (Fig. 2 B vs. Fig. 1 C). Neither MT1-MMP silencing, the addition of the pan-specific MMP inhibitor GM6001, nor the inclusion of a broad-spectrum cocktail of proteinase inhibitors (Wolf et al., 2003a) exert inhibitory effects on either HT-1080 or MDA-MB-231 cell invasion (Fig. 2, B and C).

Type I collagen telopeptides potentially regulate invasion by either affecting the linear or lateral stages of the fibrillogenic process, or by supporting collagen cross-link formation (Gelman et al., 1979; Woodley et al., 1991; Christiansen et al., 2000; Sato et al., 2000). Intact collagen can be acid-extracted in almost pure form from tissues of young animals because a subset of the lysyl oxidase-catalyzed, collagen cross-links formed in vivo are acid-labile, Schiff base adducts (Fig. 2 A) (Eyre et al., 1984). At low pH these cross-links are reversibly broken, resulting in the formation of aldehyde-bearing collagen molecules that are fully soluble in low ionic strength, acidic buffers (Fig. 2 A) (Eyre et al., 1984). The Schiff base adducts that interconnect collagen bundles are reformed spontaneously when collagen gels are reconstituted at neutral pH, but this cross-linking process can be prevented by chemically reducing the aldehyde moieties before gel formation (Fig. 2 A) (Gelman et al., 1979). When HT-1080 cells are embedded in 3D gels of aldehyde-reduced type I collagen, the cells exhibit the more rapid speed of infiltration observed in pepsin-extracted collagen gels and neither MT1-MMP silencing nor the addition of GM6001 significantly inhibits HT-1080 or MDA-MB-231 invasion (Fig. 2, B and C). As the pore size of collagen gels prepared from intact and pepsin-extracted collagen are similar (Demou et al., 2005), we conclude that MT1-MMP–independent invasion only proceeds when the structural pores formed in collagen gel networks are no longer stabilized by the covalent cross-links that define fibril architecture and structural rigidity (Zaman et al., 2006).

The precise architecture of fibrillar networks assembled in vivo cannot be duplicated fully in vitro.

Crossing an authentic stromal barrier: the human mammary gland interstitium

Reconstituted gels of native type I collagen provide important insights into the structural and mechanical properties that regulate cancer cell behavior, but the precise architecture of the fibrillar networks assembled in vivo cannot be duplicated fully in vitro (Christiansen et al., 2000; Raub et al., 2007, 2008). To define the relative roles of protease-dependent and -independent invasion programs in live tissue, we implanted MDA-MB-231 breast cancer cell spheroids into explants of the normal human mammary gland, and transplanted the composites atop the live chick chorioallantoic membrane (Sabeh et al., 2004). In this xenograft model, cancer cell spheroids embedded within the mammary gland rapidly infiltrate the surrounding tissues during a 72-h culture period (Fig. 3 A). Although both MMP-1 and MMP-2 can modulate breast cancer behavior in vivo (Tester et al., 2004; Wyatt et al., 2005; Gupta et al., 2007), silencing both proteases in tandem does not affect MDA-MB-231 or SUM-159 invasion (Fig. 3 B). MT1-MMP knockdown, in contrast, potently inhibits the ability of the breast cancer cells to infiltrate or degrade surrounding breast tissue (Fig. 3, A and B). The inhibitory effects exerted by the human-specific MT1-MMP siRNA are reversed by expressing mouse MT1-MMP in the siRNA-treated MDA-MB-231 or SUM-159 cells (Fig. 3, A and B). Hence, MT1-MMP not only supports 3D cancer cell invasion through reconstituted gels of cross-linked collagen, but also within the stromal environment of the mammary gland.

3D matrix architecture defines the mode of cancer cell invasion

In reconstituted gels of native type I collagen, pore size is limited to ~1 µm in diameter (Demou et al., 2005). The motile activity of MT1-MMP–inhibited spheroids into collagen pores was restricted under our experimental conditions to extensions of filopodia-like structures or motile cell fragments (Mayer et al., 2004). Thus, the network of rigid pores stabilized by covalent cross-links between collagen fibrils pose a significant structural constraint to the mobility of cancer cells. Current evidence suggests that a migrating cancer cell has limited ability to mechanically negotiate a rigid pore whose diameter is significantly smaller than that of the cell's nucleus, the largest and most rigid of its intracellular organelles (Lutolf et al., 2003; Demou et al., 2005; Lutolf and Hubbell, 2005; Nakayama et al.,

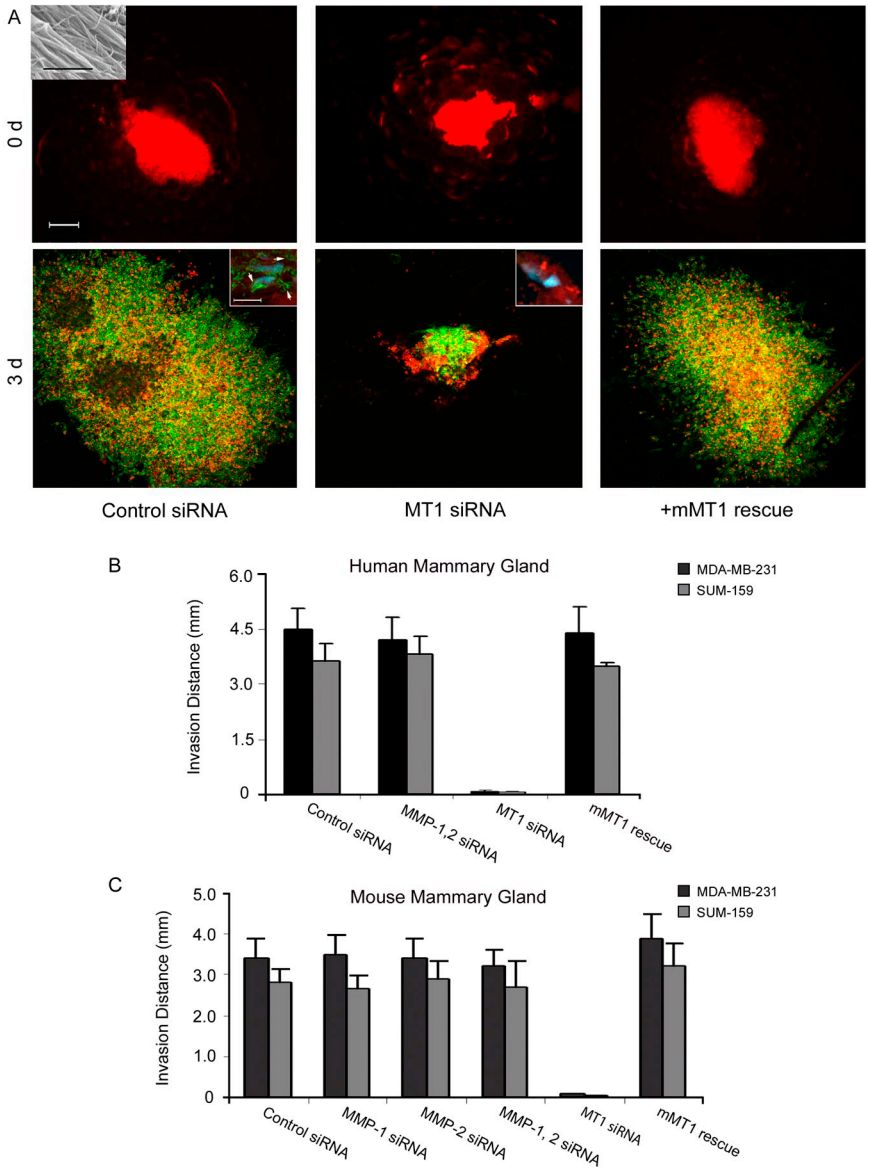


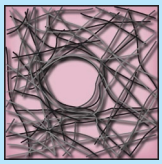
Figure 3. **MT1-MMP drives cancer cell invasion in human mammary gland explants.** (A) MDA-MB-231 Di-I–labeled spheroids (comprised of cells electroporated with a control siRNA, MT1-MMP siRNA or MT1-MMP siRNA together and a mouse MT1-MMP expression vector) were injected (30-gauge needle) into human mammary gland explants (6 × 8 x 6 mm) and the position of the cancer cell aggregates recorded by fluorescence microscopy at 0 d. Bar = 20 µm. Inset shows SEM of type I collagen fibrils in mammary gland explant. Bar = 5 µm. The inoculated explants were then cultured atop the live chick CAM for 3 d. Invasive behavior of MDA-MB-231 cells was monitored by fluorescence microscopy after labeling of mammary gland tissues with Alexa-488 phalloidin and DAPI. Bar = 200 µm. Immunofluorescent staining of mammary tissue cross sections at d 3 (inserts) reveal tracts of denatured collagen detected with monoclonal antibody HU177 (Hotary et al., 2003; Sabeh et al., 2004) (green; arrows) surrounding invasive tumor cells (red), but not MT1-MMP–silenced cancer cells (bottom panels; bar = 10 µm). (B) Invasion distance for MDA-MB-231 or SUM-159 spheroids (electroporated with control siRNA, MMP-1 and MMP-2 siRNAs, MT1-MMP siRNA or MT1-MMP siRNA in combination with a mouse MT1-MMP expression vector) was quantified after a 3-d culture period in human mammary gland explants as described. Results are expressed as mean ± SEM (n = 3). (C) Invasion distance for MDA-MB-231 or SUM-159 spheroids (electroporated with control siRNA, MMP-1 and MMP-2 siRNAs, MT1-MMP siRNA or MT1-MMP siRNA in combination with a mouse MT1-MMP expression vector) was quantified after a 3-d culture period in mouse mammary gland explants as described. Results are expressed as mean ± SEM (n = 3).

2005; Raeber et al., 2005, 2007; Ehrbar et al., 2007; Wolf and Friedl, 2008). As collagen gel microstructure can be affected by temperature, pH, ionic strength, ion stoichiometry, and monomer concentration (Roeder et al., 2002; Raub et al., 2007, 2008), minor but significant changes in experimental conditions could affect results obtained by different laboratories. However, fiber diameters generated under our standard in vitro conditions (~60 nm as assessed by SEM) are similar to those deposited at tumor sites in vivo (Oldberg et al., 2007). Further, the pore sizes generated under our in vitro conditions are consistent with the recent demonstration that 100-nm beads injected into type I collagen-rich tissues in vivo fail to diffuse significant distances from the injection site (McKee et al., 2006; Nagano et al., 2008). Though a subset of studies describing cancer cell amoeboid activity have used acid-extracted collagen, the degree of collagen cross-linking or pore size was not assessed (Wyckoff et al., 2006). These caveats aside, type I collagen autoassembly in vitro and cell-mediated assembly in vivo are mechanistically distinct processes (Raub et al., 2007, 2008). In vivo, collagen fibrils can be larger in size, incorporate a variety of other ECM components, and display distinct mechanical properties (Raub et al., 2007, 2008). Therefore, due caution should be exercised when interpreting data from in vitro assays using reconstructed collagen matrices. Nevertheless, breast cancer cells embedded in human mammary gland explants remain reliant on MT1-MMP–dependent proteolysis, leading us to conclude that carcinoma cells migrating into physiologically relevant tissues are confronted with matrix barriers similar to those generated in vitro from full-length, covalently cross-linked fibrils of type I collagen.

Caution should be exercised when interpreting data from in vitro assays using reconstructed collagen matrices.

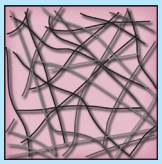
Cancer cells in vivo: never say never…

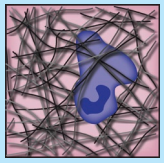
A requirement for MT1-MMP activity during stromal invasion in vitro or ex vivo contrasts with recent reports documenting amoeboid movement of breast cancer cells in the in vivo setting, particularly within the mouse mammary gland (Wyckoff et al., 2006, 2007; Xue et al., 2006; Sahai, 2007; Sahai et al., 2007). The density of collagen fibrils in the mouse mammary gland is lower than that displayed in humans (Parmar and Cunha, 2004), but we can likewise document a requirement for MT1-MMP in mouse explants (Fig. 3 C). In vivo models of real-time movement, however, monitor the short-term trafficking of cancer cells (i.e., observation periods ranging from 20 min to 4 h) from primary sites established 1–3 months in advance of microscopic analyses (Sahai, 2007; Sanz-Moreno et al., 2008). Under these conditions, rapid motility and protease-independent, amoeboid-like activity might be accommodated under specific circumstances.



Cancer cells could display rapid, protease-independent cell movement in vivo by co-opting preexisting matrix tunnels generated by tumor-infiltrating fibroblasts, smooth muscle cells, or endothelial cells that bore through tissues in an MT1-MMP–dependent process (Hiraoka et al., 1998; Chun et al., 2004; Sabeh et al., 2004; Filippov et al., 2005; Gaggioli et al., 2007). Likewise, damaged blood vessels or lymphatics as well as zones of necrosis could also present migrating cancer cells with "pre-cleared" passageways (Nagano et al., 2008). Indeed, rapid rates of cancer cell migration noted in several studies seem to occur within sites wherein the extensive loss of ECM networks has already occurred (Condeelis and Segall, 2003). In contrast, early phases of the tumor cell invasion program proceed through collagen-rich areas whose penetration would appear to be more consistent with the slower advance of proteolytically active neoplastic cell populations (Li et al., 2000; Curino et al., 2005; Provenzano et al., 2006, 2008a; Kedrin et al., 2008).

In vivo, cancer cells migrating away from the primary inoculation site potentially move into a nascent matrix deposited by infiltrating stromal cells which could contain defects in collagen cross-linking (Kauppila et al., 1998, 1999; Demou et al., 2005). Protease-independent invasion programs may, therefore, be accommodated by an immature, wound-like environment at sites distant from the neoplastic point-of-origin.





Given that only small numbers of carcinoma cells display rapid movement in vivo (Wyckoff et al., 2006, 2007; Xue et al., 2006; Sahai, 2007; Sahai et al., 2007), the issue arises as to the precise origin of this cell type. Cancer cell–macrophage hybrids have been characterized that may acquire the potential to access the myeloid-specific amoeboid phenotype (Vignery, 2005; Pawelek and Chakraborty, 2008). Indeed, cancer cell populations used in our studies are only able to traverse cross-linked type I collagen barriers by mobilizing MT1-MMP, even when collagen fiber pore size is increased from ~8 μm^2 to 80 μm^2 by decreasing the polymerization pH of the collagen gels from pH 8.5 to pH 5.5 (Raub et al., 2008) (Fig. 4, A and B). In contrast, myeloid cells such as polymorphonuclear leukocytes, T cells, and monocytes, can rapidly infiltrate identical matrices without altering collagen architecture or structure via a process resistant to MMP-specific or broad-spectrum protease inhibitors (Fig. 4 A) (Huber and Weiss, 1989; Mandeville et al., 1997; Wolf et al., 2003b; Lammermann et al., 2008). Should cancer cell–myeloid cell hybrids acquire a similar ability to traffic through collagen networks without mobilizing proteolytic machinery—an as yet unconfirmed potential—neoplastic cells may prove able to access MT1-MMP–independent programs to negotiate matrix barriers.

In the in vivo setting, caution must be exercised in assuming that ECM pores of sufficient size or structural malleability do not exist at select sites in vivo that accommodate protease-independent trafficking (Even-Ram and Yamada, 2005). For example, cancer cell trafficking within lymph nodes likely occurs through stromal cell–lined conduits that support rapid movement through matrix-free zones (Bajenoff et al., 2006). Further, tissues devoid of interstitial collagen networks, e.g., the brain (Pluen et al., 2001), may well be traversed by tumor cells without necessitating proteolytic remodeling while still requiring ROCK-regulated molecular motors to squeeze a migrating cell through matrix openings smaller than the short axis of its nucleus (Beadle et al., 2008). Nevertheless, our findings with the normal adult mammary gland, as well as those we described previously using human dermal explants (Rosenthal et al., 1998; Sabeh et al., 2004; Hotary et al., 2006), support a model wherein the type I collagen architecture of the normal interstitium presents itself as a structural barrier to cancer cell traffic.

Extending the *caveat emptor* of using cross-link–free matrix barriers

Independent of the mechanisms used by cancer cells to traverse stromal barriers, increasing attention has also focused on the reported ability of cancer cells to undergo a mesenchymal-amoeboid transition as they cross the basement membrane, a specialized ECM barrier that surrounds or underlies epithelial cells, muscle, nerves, and the endothelium (Wang et al., 2003; Zaman et al., 2006; Gadea et al., 2007; Kitzing et al., 2007; Sahai, 2007; Wolf et al., 2007; Fackler and Grosse, 2008; Rowe and Weiss, 2008). However, conclusions regarding proteinase-independent, amoeboid-type trafficking across basement membranes have been similarly based on artificial ECM composites such as Matrigel, a noncovalently cross-linked extract of basement membrane macromolecules whose structural organization fails to recapitulate that observed in vivo (Even-Ram and Yamada, 2005; Hotary et al., 2006; Rowe and Weiss, 2008; Sodek et al., 2008). Using authentic basement membranes recovered from mouse or human tissues, cancer cells likewise use MT1-MMP activity to traverse these cross-linked barriers (Hotary et al., 2006).

What's more to be done?

At the end of the day, it is unlikely that the complex milieu of the in vivo environment can be duplicated fully in vitro. Changes in oxygen tension, pH, contact guidance, and cancer cell–stromal cell crosstalk, as well as the growth factor/cytokine milieu within this microenvironment could induce changes in cancer cell behavior that are unique to the neoplastic site (Anderson et al., 2006; Provenzano et al., 2008b; Doyle et al., 2009). Dissecting the relative role of protease-dependent and -independent trafficking mechanisms in vivo will require the marriage of sophisticated 4D intravital

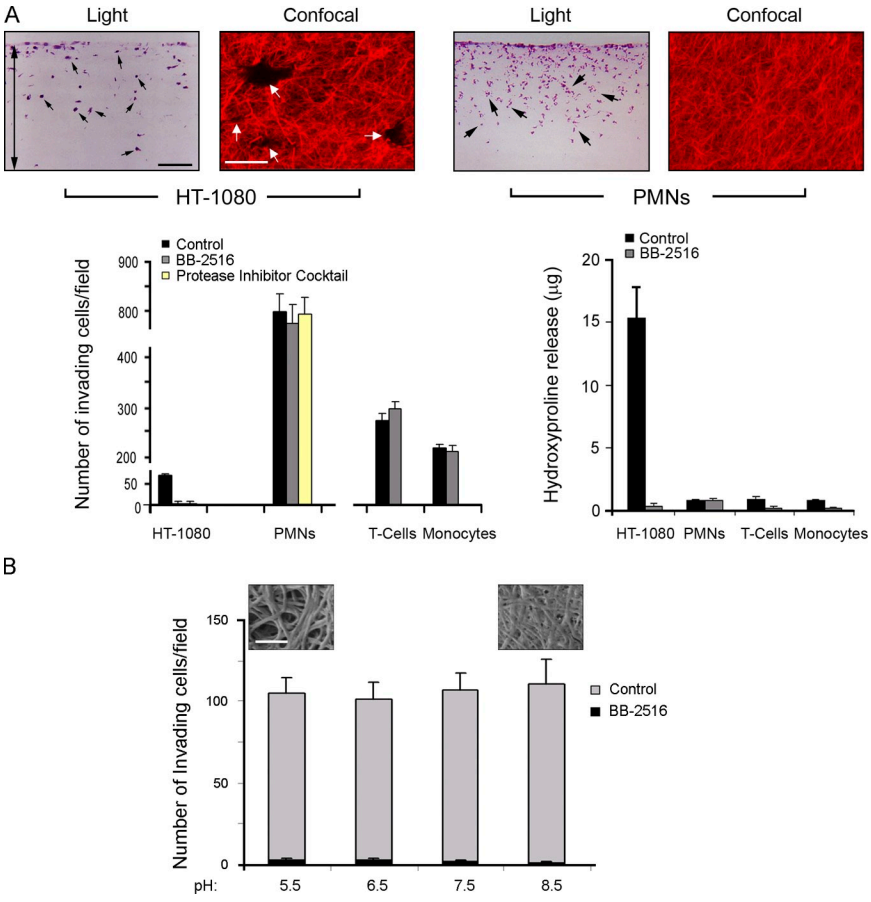


Figure 4. **Collagen-invasive and degradative activities of cancer cells versus leukocytes.** Light micrographs of cross sections of type I collagen gels (2.2 mg/ml) traversed by HT-1080 or human PMNs prepared as described (Huber and Weiss, 1989) for 3 d or 1 d, respectively. Collagen-invasive cells are H&E stained and marked with black arrows. Double-headed arrow marks the boundaries of the underlying collagen gel. Black bar = 100 µm. Laser confocal micrographs of HT-1080 cells cultured atop 3D gels of rhodamine-labeled type I collagen for 3 d demonstrate that invasion is associated with the formation of well-demarcated tunnels (white arrows; white bar = 50 µm). In contrast, PMNs stimulated with zymosan-activated plasma (Huber and Weiss, 1989) invade rhodamine-labeled collagen gels without perturbing matrix architecture (far right-hand panel). Invasion and collagen-degradative activities of HT-1080, PMNs, T cells, and monocytes (Reddy et al., 1995) were quantified in the absence or presence of BB-2516 (1.5 µM) as described previously (bar graphs, bottom panel) (Sabeh et al., 2004). PMN invasion was also assessed in the presence of the protease inhibitor cocktail prepared as described (Wolf et al., 2003a). Results are expressed as mean ± SEM (n = 4). (B) Acid-extracted, rat tail collagen gels were prepared at pH 5.5, 6.5, 7.5, and 8.5 as described (Raub et al., 2008) and the invasive activity of HT-1080 cells into the 3D gels was quantified in the absence or presence of BB-2516 (1.5 µM) after a 3-d culture period. Collagen at pH 5.5 and 8.5 as visualized by SEM are shown in the insets (n = 3; bar = 250 nm).

microscopic techniques with conditional knockout models and/or the selective silencing of MT-MMP family members in primary populations of human cancer cells embedded within the humanized mouse mammary gland (Proia and Kuperwasser, 2006). Although such studies are likely forthcoming (Alexander et al., 2008; Kedrin et al., 2008), a reductionist's approach to modeling cancer cell invasion in vitro suggests that the frequently assumed "given" of cancer cell mesenchymal-amoeboid transitions that allow for protease-independent invasion must be viewed cautiously as investigators attempt to extend current insights into the in vivo setting. Indeed, when confronting rigid structural barriers whose pore size is not permissive for passive cell movement, cancer cells appear to be almost entirely reliant on MT1-MMP activity, and perhaps the closely related membrane-anchored collagenase MT2-MMP (Hotary et al., 2000, 2006). Certainly, a "one size fits all" model—even regarding the dominant role proposed for MT1-MMP in tumor cell invasion—seldom stands the test of time when applied to the complex in vivo environment. No doubt, conditions will exist in vivo where the cancer cell will use actomyosin-generated forces to either displace impeding barriers or alter its nuclear dimensions to "squeeze" through narrow pores. However, as important roles for MT-MMP family members in cancer invasion, growth, and systemic spread continue to be defined (Taniwaki et al., 2007; Hu et al., 2008; Husemann et al., 2008; McGowan and Duffy, 2008; Szabova et al., 2008; Devy et al., 2009), carefully controlled in vitro and in vivo models should allow for new insights into the mechanisms controlling cancer cell migration through the ECM.

A "one size fits all" model seldom stands the test of time when applied to the complex in vivo environment.

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