Shedding of RANKL by Tumor-Associated MT1-MMP Activates Src-Dependent Prostate Cancer Cell Migration

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Abstract

Membrane type 1 matrix metalloproteinase (MT1-MMP) plays an essential role in protease-mediated extracellular matrix (ECM) degradation, but it also functions as a sheddase releasing non-ECM substrates such as receptor activator of NF-κB ligand (RANKL), an osteoclastogenic factor typically confined to the surface of osteoblasts. We previously found high expression of MT1-MMP in skeletal metastasis of prostate cancer patients, in a pattern similar to RANKL expression. We also showed that overexpression of MT1-MMP in prostate cancer cells increases tumor growth and osteolysis in an intratibial mouse model of bone metastasis, and that soluble factor(s) shed by tumor-derived MT1-MMP enhance osteoclast differentiation in a RANKL-dependent manner. Recent evidence indicates that the cognate receptor for RANKL, RANK, is expressed in prostate cancer cells, suggesting the presence of an autocrine pathway. In this study, we show that MT1-MMP–expressing LNCaP prostate cancer cells display enhanced migration. Moreover, conditioned medium from LNCaP cells expressing both RANKL and MT1-MMP stimulates the migration of MT1-MMP–deficient C42b prostate cancer cells. This enhanced chemotaxis can be abrogated by osteoprotegerin (soluble decoy receptor of RANKL), MIK-G2 (a selective inhibitor for MT1-MMP), and PP2 (a Src inhibitor). These findings indicate that tumor-derived MT1-MMP enhances tumor cell migration through initiation of an autocrine loop requiring ectodomain shedding of membrane-bound RANKL in prostate cancer cells, and that Src is a key downstream mediator of RANKL-induced migration of prostate cancer cells. Cancer Res; 70(13); OF1–9. ©2010 AACR.

Introduction

Disseminated tumor cells must proteolytically degrade and then invade the surrounding extracellular matrix (ECM) to expand into a large metastatic deposit (1, 2). In bone metastasis, tumor cells must invade through type 1 collagen, the main organic component of interstitial ECM. Matrix metalloproteinases (MMP) are well known to play a significant role in ECM remodeling (3–5). Membrane type 1 MMP (MT1-MMP), in particular, plays a critical role in type 1 collagen turnover, as shown by skeletal defects seen in MT1-MMP–deficient mice (6). Furthermore, MT1-MMP has been shown to degrade type 1 collagen directly (7), and it is essential for tumor cell invasion through three-dimensional type 1 collagen matrices (8). Previously, we showed a heterogeneous pattern of MT1-MMP expression in carcinoma glands within the prostate of patients undergoing radical prostatectomy (9). In contrast, we found a homogeneous and intense expression by cancer cells within human prostate cancer bone metastases from a “rapid autopsy” program (10). Moreover, we found that prostate cancer cells ectopically expressing MT1-MMP display increased tumor growth and tumor-induced osteolysis in an intratibial mouse model of bone tumor growth (10).

Typically expressed on the surface of cells of the osteoblast lineage, the receptor activator of NF-κB ligand (RANKL) is a potent inducer of osteoclast maturation (11). RANKL is a homotrimeric transmembrane complex that can be shed from the cell surface through MMP-mediated proteolytic cleavage (12–14). Among the members of the MMP family, MT1-MMP is the most efficient sheddase of RANKL (15). In fact, knockdown of endogenous MT1-MMP proved to be sufficient to diminish levels of soluble RANKL in vitro and in vivo (16). Interestingly, recent reports show that RANKL is also expressed by prostate cancer cells in human bone metastasis (17–21) and in several human prostate cancer cell lines (22, 23). Zhang and colleagues showed that prostate cancer cells release soluble RANKL, promoting in vitro osteoclastogenesis independent of osteoblasts or bone stromal cells (22). These data suggest a role for tumor-derived RANKL in mediating some of the bone responses seen in metastatic prostate cancer. We subsequently found that conditioned media (CM) derived from prostate cancer cells...
expressing both RANKL and MT1-MMP enhanced in vitro differentiation of osteoclasts, an effect blocked by either osteoprotegerin or a selective MT1-MMP inhibitor (10). These data highlight a mechanism for metastatic tumor expansion wherein tumor-associated MT1-MMP acts as a mediator of autocrine/paracrine signaling through solubilization of RANKL. Of course, this does not rule out direct degradation of the ECM by MT1-MMP.

Consistent with the "osteomimicry" theory (24–26), recent evidence indicates that RANK, the cognate receptor for RANKL, is also found on the surface of prostate cancer cells (27, 28). Activation of RANK in prostate tumor cells is associated with increased cell migration, invasion through a collagen matrix, stimulation of mitogen activated kinases (MAPK), and augmented expression of osteoclast-related genes (27–29). Based on these considerations, we hypothesized that MT1-MMP may play a role in RANK activation and subsequent migration in tumor cells. Herein we show that tumor-associated MT1-MMP promotes tumor cell migration through a novel indirect mechanism involving solubilization of RANKL by tumor-associated MT1-MMP and subsequent autocrine activation of RANK. RANK-mediated migration proceeds through rapid activation of a Src-dependent pathway. This MT1-MMP/RANKL/RANK/Src axis may have important implications for the treatment of prostate cancer bone metastasis.

Materials and Methods

Cell culture

LNCaP and PC3 cells, obtained from American Type Culture Collection (ATCC), were maintained in RPMI 1640. C42b cells, an LNCaP variant isolated from castrated mice with preferential growth in bone (courtesy of Dr. Leland Chung, Emory University, Atlanta, GA; ref. 30), were maintained in T-medium. DU145 cells were obtained from ATCC and maintained in DMEM. All culture media were purchased from Invitrogen and supplemented with 10% fetal bovine serum (FBS). Pooled populations of LNCaP cells with ectopic expression of wild-type MT1-MMP (LNCaP-MT1) or control (LNCaP-Neo) were established and maintained as previously described (10). Cell lines obtained from ATCC are routinely karyotyped, identity verified using short tandem repeat profiling analysis, and contamination checks.

Immunoblot analysis

LNCaP, C42b, PC3, DU145, or LNCaP transfectants were cultured to 80% to 90% confluency. Whole-cell lysates were resolved on 10% SDS-polyacrylamide gels or 4% to 12% Bis-Tris gradient gels (Invitrogen) under reducing conditions, and immunoblotted with antibodies targeted at the catalytic domain of human MT1-MMP (LEM-2/15 monoclonal antibody kindly provided by Dr. A. Arroyo, Hospital de la Princesa, Madrid, Spain), RANKL (R&D Systems), RANK (Cell Signaling Technology), or osteoprotegerin (R&D Systems). Immunoreactive proteins were detected with horseradish peroxidase–conjugated anti-mouse IgG or anti-rabbit IgG antibodies, and enhanced chemiluminescence (Pierce). Blots were stripped and reprobed with an antibody to β-actin (Sigma-Aldrich), used as loading control. Western blots were repeated under independent conditions at least twice; representative blots are shown.

Transwell migration assay

Serum-starved C42b cells (5 × 10⁴) were allowed to migrate for 16 hours through noncoated 8-μm-pore Transwell inserts (BD Falcon) toward human recombinant RANKL (rRANKL; Leinco Technologies, Inc.) or protein-normalized 48-hour CM obtained from LNCaP-Neo or LNCaP-MT1 cells (40% in culture medium at a final FBS concentration of 0.5%). Cells that migrated through the filters were counted in five randomly picked 200× fields, or in the entire filter if the number of cells was low. Data were expressed as mean ± SEM of average number of cells obtained in each filter (n ≥ 3), with experiments repeated under independent conditions at least twice. For migration studies under Src inhibition, cells were treated 45 minutes before the assay and during the assay with 5 μmol/L 4-amino-5-(4-chlorophenyl)-7-[(t-buty)prazolo][3,4-d]pyrimidine (PP2). To inhibit RANKL-induced migration, RANKL or CM was incubated together with osteoprotegerin for 45 minutes at 37°C before insertion of upper chamber containing cells into the 24-well plate. In experiments aimed at inhibiting the activity of tumor-associated MT1-MMP, LNCaP-Neo and LNCaP-MT1 cells were incubated for 48 hours with 10 μmol/L 4-[4-(methanesulfonamido)phenoxyl]phenylsulfonyl methylthiirane (MIK-G2; ref. 31), an MT1-MMP inhibitor, before collection of CM.

Experiments using LNCaP-transfectants alone were performed as described above, except that no chemoattractant was used.

In vitro cell viability assessment

C42b cells were incubated with different concentrations of recombinant RANKL, osteoprotegerin, PP2, or MIK-G2 for 16 hours to parallel the conditions used in the migration assays. Cell viability was measured using the WST-1 assay, following manufacturer’s instructions (Roche Applied Science), and the trypan blue exclusion test.

Signaling studies

C42b cells at 90% confluency were serum starved for 18 hours and then treated with 200 ng/ml rRANKL in serum-free T-medium for different lengths of time or increasing concentrations of rRANKL. Whole-cell lysates were separated by SDS-polyacrylamide gels as described above. The following primary antibodies from Cell Signaling Technology were used: anti-phospho-Src (Y419), total Src (L4A1), polyclonal phospho-Akt (Ser473); polyclonal total Akt; monoclonal phospho-p44/42 MAPK (Thr202/Tyr204; 197G2); polyclonal total p44/p42 MAPK; polyclonal phospho-stress-activated protein kinase/c-jun-NH₂-kinase (SAPK/JNK; Thr183/Tyr185); polyclonal total SAPK/JNK; polyclonal phospho-p38 MAPK (Thr180/Tyr182); and polyclonal total p38 MAPK. The antigen-antibody complexes were detected as described above. For studies in
which Src was inhibited, cells were pretreated for 45 minutes in the presence of different concentrations of PP2, and then treated with 200 ng/mL of rRANKL in the presence of PP2. Studies of phosphorylated and total Src were also performed in PC3 or LNCaP-transfectant cells as described above. Western blots were repeated under independent conditions at least twice; representative blots are shown.

Confocal microscopy

C42b cells grown in eight-well chamber Labtek II CC2 slides (NUNC) were serum starved for ~18 hours, and then treated with 200 ng/mL of rRANKL for 5 minutes or with vehicle control. Cells were fixed with 4% paraformaldehyde, permeabilized, and then incubated with fluorescent-labeled phalloidin conjugate (Millipore). After mounting with anti-fade Vectashield Mounting Media with 4′,6-diamidino-2-phenylindole (DAPI; nuclear stain; Vector Laboratories, Inc.), cells were examined on a Zeiss LSM510 confocal microscopy system (Carl Zeiss, Inc.) at 63× magnification.

Statistical analysis

Differences among more than two groups were analyzed by one-way ANOVA with Tukey-Kramer posttesting. Data comparing differences between two groups were statistically analyzed using unpaired Student’s t test. Differences were considered significant when $P < 0.05$.

Results

LNCaP cells with ectopic expression of MT1-MMP display increased motility

To examine a role for tumor-derived MT1-MMP independent of ECM proteolytic degradation, we examined the effect of ectopic expression of MT1-MMP in prostate cancer cells in the absence of a restrictive ECM. LNCaP transfectants with differential expression of ectopic MT1-MMP previously established by us (10) were assayed for cell motility in the absence of any chemoattractant. Notably, the number of LNCaP-MT1 cells traversing the pored filters was three times as many as that of control LNCaP-Neo cells (Fig. 1) despite comparable doubling times (10) and cell survival (data not shown), suggesting an intrinsic motile response.

CM from LNCaP cells transfected with wild-type MT1-MMP stimulates migration of C42b cells in an MT1-MMP- and RANKL-dependent manner

Given the ability of tumor-associated MT1-MMP to shed RANKL from the cell surface (10), we evaluated the possibility that the motility of MT1-MMP–expressing LNCaP cells was due to autocrine activation of RANK by tumor-derived soluble RANKL. To test this hypothesis, C42b cells were chosen as “reporter” cells because, like LNCaP cells, they express RANK but not MT1-MMP or osteoprotegerin (Fig. 2A). We found that CM derived from LNCaP-MT1 cells significantly enhanced C42b cell migration compared with control LNCaP-Neo–derived CM (Fig. 2B). Moreover, this migration was significantly reduced when the medium was conditioned in the presence of MIK-G2, a selective MT1-MMP inhibitor, suggesting a role for MT1-MMP activity in the generation of the soluble factor responsible for the migratory response.

Given that the LNCaP transfectants express RANKL (10), similar to the other human prostate cancer cell lines tested by us (Fig. 2A), we asked whether the enhanced migratory response of C42b cells was caused by soluble RANKL present in the CM of LNCaP-MT1 cells. Therefore, cell migration assays were conducted with CM in the presence or absence of osteoprotegerin, a natural decoy receptor that competes with RANK for RANKL. As can be seen in Fig. 2C, the presence of osteoprotegerin significantly reduced the ability of the LNCaP-MT1–derived CM to stimulate the migration of C42b cells, reducing the total number of migrated cells to levels observed with media derived from LNCaP-Neo cells. Conversely, preincubation of LNCaP-Neo–derived CM with osteoprotegerin did not alter the baseline cell migration rates of C42b cells. These results suggest that increased migration of the C42b cells was due to the presence of soluble RANKL in the CM of LNCaP-MT1 cells.

We next sought to determine the chemotactic effect of rRANKL on C42b cells. We observed a dose-dependent
increase in C42b cell migration (Fig. 3A) in response to soluble rRANKL, which was abrogated by preincubation with increasing concentrations of osteoprotegerin in a dose-dependent manner (Fig. 3B). This result is consistent with the ability of CM derived from LNCaP-MT1 cells to stimulate cell migration in an osteoprotegerin-sensitive manner, and it directly implicates RANK activation by soluble RANKL (Fig. 2C). Furthermore, we confirmed that RANKL has no mitogenic effect on C42b cells (data not shown). Therefore, the increased number of cells traversing the filter was due to migration.

**RANKL activates Src in a dose-dependent manner and initiates cytoskeletal rearrangement**

Next, we examined downstream signaling as a consequence of RANK activation in C42b cells in response to soluble rRANKL. RANK lacks intrinsic kinase activity, requiring the recruitment of tumor necrosis factor receptor–associated factors (TRAF) to the cell membrane for downstream signaling (32). Therefore, we looked downstream of TRAF binding to find a sensitive end point for RANK activation. We found that treatment of C42b cells with 200 ng/mL of rRANKL induced activation of multiple signaling pathways (Fig. 4A). Of all the intracellular signaling molecules we studied, Src and Erk showed the strongest and most rapid activation. Src phosphorylation of tyrosine 419 was observed within 15 minutes of exposure to rRANKL (Fig. 4A), followed by a reduced activation at 30 minutes, whereas Erk phosphorylation was noticeable between 15 and 30 minutes after treatment with rRANKL. Although Src has been reported to be an upstream activator of Akt in osteoclasts (33), we did not see any effect on phosphorylated Akt levels in rRANKL-treated C42b cells. As previously reported (28), we found that RANK activation by rRANKL also promoted activation of MAPKs (Erk1/2, p38 MAPK, and JNK/SAPK).

Based on a potential “osteomimetic” parallel between Src activation in C42b prostate cancer cells by rRANKL and osteoclast dependence on Src activity, we focused on Src activation in prostate cancer cells upon activation by RANKL. We observed that rRANKL had a dose-dependent effect on Src phosphorylation (Fig. 4B, top), attesting to the specificity of rRANKL on regulation of Src. rRANKL consistently increased phosphorylation of tyrosine 419, the autophosphorylation site located within the Src catalytic domain, within 15 minutes of exposure to exogenous rRANKL, actually peaking at 5 minutes (Fig. 4B, bottom). This rapid activation of Src is consistent with previous studies showing that Src is one of the immediate signaling molecules activated by the RANKL/RANK signaling axis (34). PC3 cells, which also express RANK (Fig. 2A), also responded to RANKL treatment by activating Src, showing that this effect is not specific to C42b cells (Fig. 4C). Based on our observation that rRANKL increased C42b cell migration, and the reported role of Src as a mediator of cellular migration (27, 29), we examined cytoskeletal rearrangements in C42b cells. TRITC-phalloidin staining of F-actin in C42b cells treated for 5 minutes with 200 ng/mL of rRANKL revealed...
actin polymers beneath the plasma membrane, compared with control (Fig. 4D).

rRANKL-induced C42b cell migration is dependent on Src activity

To establish the role of Src in rRANKL-induced C42b migration, we used the specific Src inhibitor PP2, which binds active Src within the ATP binding pocket inhibiting autoactivation (35). Exposure of C42b cells to increasing concentrations of PP2 inhibited the ability of rRANKL (200 ng/mL for 5 minutes) to induce Src phosphorylation (Fig. 5A). At 1.25 μmol/L PP2, levels of active Src were decreased, and at 2.5 μmol/L PP2, phosphorylation at tyrosine 419 of Src was not detectable by immunoblotting. Concurrent examination of MAPKs revealed that PP2 did not affect RANKL-induced activation of these kinases, suggesting that the RANKL-RANK-Src pathway is independent of the RANKL-RANK-MAPK pathway. To implicate Src activation in RANKL-induced migration, we next analyzed chemotaxis of C42b cells toward rRANKL in the presence of PP2. As can be seen in Fig. 5B, PP2 inhibited C42b cell migration in a dose-dependent fashion. Of note, at these concentrations, PP2 did not affect C42b cell viability (data not shown).

MT1-MMP expression by LNCaP cells stimulates cell migration in a Src-dependent manner

To this point, we showed only the effects of rRANKL on inducing Src-dependent cell migration in prostate cancer cells. It was therefore crucial to determine if CM from prostate cancer cells expressing MT1-MMP and RANKL produced these same effects. We first examined the basal levels of Src activity in our LNCaP-Neo and LNCaP-MT1 cells. By Western blot, we observed an increase in phosphorylated Src in LNCaP-MT1 cells versus LNCaP-Neo (Fig. 6A), suggesting a correlation between tumor-associated MT1-MMP expression/activity and increased Src activity. We then examined the ability of LNCaP transfectant–derived CM to activate Src in C42b cells. Serum-starved C42b cells were treated for 15 minutes with protein-normalized CM from LNCaP-Neo and LNCaP-MT1 cells. We found that C42b treatment with LNCaP-MT1–derived CM increased phosphorylated Src levels relative to LNCaP-Neo derived CM (Fig. 6B). Moreover, enhanced Src activation caused by LNCaP-MT1 CM was hindered by pretreatment of C42b cells with 5 μmol/L PP2, confirming effective Src kinase inhibition (Fig. 6B). To confirm that Src activation was due to RANKL signaling by soluble RANKL present in LNCaP-MT1–derived CM, we preincubated the CM with an excess of osteoprotegerin. As can be seen in Fig. 6B, preincubation with osteoprotegerin resulted in considerably reduced levels of phosphorylated Src in C42b treated with LNCaP-MT1–derived CM.

We next sought to confirm that increased Src activation in C42b, as a result of treatment with LNCaP-MT1–derived CM, was responsible for the enhanced migration displayed by C42b cells, similarly to rRANKL. C42b cells were pretreated with 5 μmol/L of the Src inhibitor PP2, and then assayed for cell migratory ability toward LNCaP-MT1– or LNCaP-Neo–derived CM. The enhanced chemotactic response of C42b toward LNCaP-MT1–derived CM was significantly inhibited by PP2, whereas the migratory response to LNCaP-Neo–derived CM remained unchanged despite the presence or absence of the Src inhibitor (Fig. 6C). These data suggest that Src activity is a requirement for C42b migration in response to LNCaP-MT1–derived CM.

Discussion

Substantial evidence supports involvement of tumor-derived MMPs in several steps of the metastatic cascade, and it is now well accepted that MMPs have roles above and beyond degradation of ECM. However, the failure of broad-spectrum MMP inhibition in clinical trials has shifted the focus to specific members of the MMP family (36, 37). Functional redundancies within the MMP family have hindered these efforts; however, the unique properties of MT1-MMP in skeletal development (6) may offer an opportunity...
for specific MMP-targeted therapy. Indeed, MT1-MMP inhibition continues to be of interest to the pharmaceutical industry (38). The role of MT1-MMP in providing a mechanism for tumor cell movement through ECM is without a doubt an important function of this protease; however, in this study, we examined an alternative role for tumor-associated MT1-MMP. Our data show the ability of tumor-derived MT1-MMP to enhance prostate cancer cell migration independent of protease-mediated ECM degradation through an MT1-MMP/RANKL/RANK autocrine pathway dependent on Src activation.

Initially, we observed increased motility of prostate cancer cells expressing MT1-MMP in an ECM-free system. We also saw RANKL-dependent enhancement of the migratory capacity of C42b cells toward CM from LNCaP cells expressing MT1-MMP. In agreement with others (27, 29), we found RANK to be expressed in all prostate cancer cells examined, and the RANK expressed was functional because we observed a specific RANKL-mediated dose-dependent increase in C42b cell migration. The use of prostate cancer cell–derived CM in our studies (in addition to recombinant RANKL) opened the possibility of multiple soluble factors having redundant effects.

Therefore, we systematically and independently inhibited MT1-MMP and RANKL to support the existence of a specific tumor-associated MT1-MMP/RANKL/RANK autocrine signaling axis. Our data supplement prior findings (27, 29) in which RANKL induced activation of MAPKs in prostate cancer cells. Based on the osteomimetic properties displayed by tumor cell types with a preference for bone metastasis, we reasoned that signaling critical for osteoclasts would also be important for cancer cells growing within the bone. In particular, Src plays a pivotal role in osteoclast RANK signaling, affecting bone structure and function (34). In fact, the only major finding in c-Src knockout mice is an osteopetrotic phenotype, despite ubiquitous Src expression in many tissues (39, 40). Therefore, we investigated RANKL-induced Src activation in prostate cancer cells. We found that chemical inhibition of Src activity significantly inhibited RANKL-induced C42b cell migration, with no effect on MAPK signaling. Additionally, LNCaP-MT1 cells displayed a constitutive increase in Src activation when compared with LNCaP-Neo. This activation was also observed in C42b cells exposed to LNCaP-MT1–derived CM, and was inhibited when the CM was preincubated with osteoprotegerin. These results clearly show an
association between tumor-associated MT1-MMP activity and soluble RANKL derived from the same cells, resulting in activation of RANK expressed on the surface of prostate cancer cells and subsequent activation of Src. Moreover, inhibition of Src by PP2 in C42b cells suppressed the enhanced migration caused by LNCaP-MT1–derived CM, implying an essential role for Src in this mechanism. To our knowledge, we are the first to show that Src is a key downstream mediator of RANKL-induced migration in tumor cells.

The novelty of our findings is based on the involvement of tumor-derived MT1-MMP in proteolytic cleavage of a non-ECM substrate to enhance prostate cancer cell migration. Recently, osteoclast-derived MMP-7 has been implicated in prostate and mammary tumor-induced osteolysis, presumably due to processing of osteoblast-derived membrane-bound RANKL to its soluble form leading to osteoclast maturation and activation (14, 41). We are the first to describe a connection between tumor-derived RANKL and tumor-associated MT1-MMP, wherein an autocrine loop in prostate cancer cells triggered by RANKL proteolytically shed from the surface of the cancer cell leads to enhanced cell migration. Furthermore, our data imply that migration induced by the MT1-MMP/RANKL/RANK axis in prostate cancer cells is dependent on Src activity.

Notably, in vivo studies performed by us with the same MT1-MMP–overexpressing prostate cancer cells used herein clearly showed an essential role of tumor-associated MT1-MMP in intraosseous growth and osteolysis (10), suggesting that MT1-MMP/RANKL/RANK–mediated migration of prostate cancer cells may also have in vivo implications on tumor expansion within bones. Of course, these findings do not exclude a role for tumor- or osteoblast-derived RANKL in stimulating osteoclasts. To address the actual role of tumor-derived RANKL and autocrine signaling, knockdown experiments are currently under way in our laboratory to suppress the expression of endogenous RANKL or RANK in prostate cancer cells with either high or absent MT1-MMP expression and activity. Using these cell variants and the intratibial model, we expect to dissect the contribution of tumor-derived soluble RANKL and autocrine RANKL/RANK signaling to intraosseous tumor expansion.

The potential clinical relevance of a MT1-MMP/RANKL/RANK pathway is supported by data from human tissues. Prior studies by us and others showed expression of MT1-MMP (10) and RANKL (17–21) in clinical prostate cancer bone metastases, as well as expression of RANK (28, 29) and Src (42, 43) in human prostate cancer cells. Moreover, in addition to the apparent trend for an enhanced expression of MT1-MMP in prostate cancer bone metastasis (10) with respect to primary prostate cancer (9) observed by us, it has recently been proposed that the interaction of prostate cancer and the bone microenvironment results in an increase in RANKL expression by prostate cancer cells (44). Figure 6D shows a model that incorporates our current data and published results, and illustrates an autocrine prostate tumor-derived MT1-MMP/RANKL/RANK pathway mediated through Src. Thus, in addition to the effects of RANKL on bone stroma, our model suggests a role for RANKL in prostate cancer cells. According to this model, active tumor-associated MT1-MMP cleaves RANKL expressed on the surface of prostate cancer cells into a soluble form of RANKL by ectodomain shedding. The binding of soluble RANKL to RANK at the membrane of prostate cancer cells signals through a pathway that involves Src activation, resulting in cytoskeletal rearrangement and increased migration of tumor cells. Inhibition of MT1-MMP activation (MIK-G2), RANKL binding, or Src phosphorylation (PP2) abrogates the enhanced migration of prostate cancer cells exposed to soluble factors produced by MT1-MMP–expressing cancer cells, suggesting that these events constitute potential targets that could be used to improve the treatment of prostate cancer.
bone metastatic disease. Recently, clinical trials with a fully human anti-RANKL antibody have been initiated in patients with prostate cancer bone metastases with promising results (45). Also, Src inhibitors are currently under clinical investigation for patients with metastatic castration-resistant prostate cancer; available data show activity in bone (46–48).

Based on our results, we propose that the use of Src inhibitors in combination with RANKL and/or selective MT1-MMP inhibitors may be of therapeutic benefit in prostate cancer patients with skeletal metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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