

Topic: How does the prostate cancer microenvironment affect the metastatic process and/or treatment outcome?

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Membrane-type matrix metalloproteinases: expression, roles in metastatic prostate cancer progression and opportunities for drug targeting

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ABSTRACT

The membrane-type matrix metalloproteinases (MT-MMPs), an important subgroup of the wider MMP family, demonstrate widespread expression in multiple tumor types, and play key roles in cancer growth, migration, invasion and metastasis. Despite a large body of published research, relatively little information exists regarding evidence for MT-MMP expression and function in metastatic prostate cancer. This review provides an appraisal of the literature describing gene and protein expression in prostate cancer cells and clinical tissue, summarises the evidence for roles in prostate cancer progression, and examines the data relating to MT-MMP function in the development of bone metastases. Finally, the therapeutic potential of targeting MT-MMPs is considered. While MT-MMP inhibition presents a significant challenge, utilisation of MT-MMP expression and proteolytic capacity in prostate tumors is an attractive drug development opportunity.

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INTRODUCTION

Several decades of research have established the matrix metalloproteinases (MMPs) as key players in the progression of cancer, largely through alterations observed in the tumor microenvironment. Indeed, MMPs have been considered as potential diagnostic

and prognostic biomarkers for many types and stages of cancer^[1]. Despite their potential as therapeutic targets, however, a clinically useful agent has yet to materialise, despite several MMP inhibitors having been developed and evaluated in clinical trials^[2]. More recently, attention has turned to the potential utility of MMPs as activators of targeted prodrug therapies^[3-5].



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Table 1: MT-MMP nomenclature

Gene	Protein
<i>MMP-14</i>	MT1-MMP
<i>MMP-15</i>	MT2-MMP
<i>MMP-16</i>	MT3-MMP
<i>MMP-17</i>	MT4-MMP
<i>MMP-24</i>	MT5-MMP
<i>MMP-25</i>	MT6-MMP

MT-MMP: membrane-type matrix metalloproteinases

The MMP family of proteolytic enzymes comprises over 26 structurally similar zinc-dependent endoproteases. The wider family comprises two major subgroups: (1) the soluble or secreted MMPs and (2) the membrane-type MMPs (MT-MMPs). The MT-MMPs are further sub-classified by their cell surface association, either by a transmembrane domain (as is the case for MT1-, MT2-, MT3-, and MT5-MMP) or by a glycosphosphatidylinositol anchor (in MT4- and MT6-MMP). The nomenclature for the MT-MMPs is outlined in Table 1: the gene and protein names are generally used interchangeably. The structures of the MT-MMP family have been well described^[6]. They are each synthesised as inactive pre-pro enzymes in the Golgi apparatus, with cleavage of the signal peptide and pro-domain required before transport to the cell surface.

The roles played by many MMPs in wider cancer initiation, progression and metastasis have been extensively explored, with pivotal roles described in prostate cancer in particular^[7]. Expression and the roles played by the MT-MMP family in prostate cancer are less well studied, however, and it is this area that is the focus of this review. The available evidence for expression of MT-MMPs in prostate cancer cells and clinical tissues will be examined first, followed by consideration of their roles in prostate cancer function and in metastasis to the bone.

EXPRESSION OF MT-MMPs IN PROSTATE CANCER CELL LINES

A significant body of literature exists concerning the expression of MT1-MMP in prostate cancer cell lines, albeit limited almost exclusively to the widely studied PC3, DU145 and LNCaP cell lines. Prostatic adenocarcinoma cells PC3 (derived from a bone metastasis) and DU145 (derived from a brain metastasis) are androgen-insensitive and metastatic (PC3 being the more aggressive of the two), while LNCaP cells (prostatic adenocarcinoma cells derived from a lymph node metastasis) are androgen-sensitive cells and non-metastatic^[8].

MT1-MMP gene expression has been consistently

reported in the androgen-insensitive, more metastatic cell lines PC3 (and sublines PC3-M and PC3-MM2^[8]) and DU145. Expression has also been reported in TSU-Pr1 cells^[9,10], also androgen-insensitive. Meanwhile, the less aggressive androgen-sensitive cell line LNCaP (and sublines LNCaP-C4 and LNCaP-C4-2^[8]) exhibit low or an absence of *MT1-MMP* gene expression^[8,10-13]. Daja *et al.*^[8] explain that despite these differences in gene expression, active MT1-MMP protein expression was identified in both LNCaP and PC3 cells and their sublines. Furthermore, Jennbacken *et al.*^[14] demonstrated that transformation of LNCaP into an androgen-independent cell line (i.e. LNCaP-19^[15]) was accompanied by increased aggressiveness (growth and migratory capacity) and by upregulation of both MT1-MMP gene and protein expression. The influence of the tumor microenvironment, specifically fibroblasts (WPF5), was investigated on PC3 and DU145 cells in a study by Coulson-Thomas *et al.*^[16]. An increase in MT1-MMP gene and protein expression was reported in co-cultures of WPF5 and PC3 or DU145 cells. Protein was localised at the cellular projections of all cell lines. When considered together with changes in vimentin distribution and an up-regulation of integrin $\alpha 5 \beta 1$ expression, this is indicative of a more invasive phenotype.

Information regarding the remaining members of the MT-MMP family is more scarce. Interestingly, in contrast to the picture observed with *MT1-MMP*, *MT2-MMP* gene expression has been reported as more significant in LNCaP cells and sublines than in PC3 cells. The pattern of *MT3-MMP* gene expression is the opposite to this, and thus similar to that observed for *MT1-MMP*^[8]. Protein levels were similar in both cell lines, however. Processed MT-MMPs, indicative of latent MMP activation, were observed to be increased in the more aggressive sublines. Jung *et al.*^[11] described significant gene expression of MT2-MMP and MT5-MMP in both cell types. MT3-MMP expression was observed in LNCaP cells with negligible expression in PC3 or DU145 cells. Meanwhile, MT4-MMP expression was observed in PC3 and DU145, with negligible expression in LNCaP cells.

EXPRESSION OF MT-MMPs IN PROSTATE CANCER CLINICAL TISSUES

The clinical expression of secreted MMPs in prostate cancer has been well reviewed by Gong *et al.*^[7], with expression of MMP-2, -3, -7, -9 and -13 gene and protein each identified in serum and tumor tissue of patients with prostate cancer, and correlation with progression and metastasis observed. Interestingly,

MMP-1 expression has been associated with lower grade prostate tumors. In this section, we have summarised the clinical expression data available for MT-MMPs in prostate cancer tissues.

Trudel *et al.*^[17] examined tissues from 189 prostate cancer patients who had undergone surgery (radical prostatectomy). MT1-MMP expression and its effects on disease-free survival were examined immunohistochemically, differentiating cancer, stromal, and benign epithelial cells. This study showed that in 167 (88.8%) cases, MT1-MMP was expressed by benign epithelial cells and MT1-MMP was expressed by cancer cells in 171 (90.5%) cases. The expression in cancerous tissue was mostly observed in cells at or near the tumor front. Overall the expression was described as heterogeneous though cancer cells at the tumor margin were seen to always express MT1-MMP. The expression of MT1-MMP in benign epithelial cells was somewhat unexpected, given what is generally understood about MMPs in other cancers. The authors did raise the issue of the quality and specificity of commercial antibodies for MT1-MMP, and did further suggest that active MT1-MMP could be cleaved by other MMPs resulting in soluble fragments, which may have been detected. It is the potential effect of prostate cancer cells on the local microenvironment that is perhaps the key, however. The authors speculated as to whether the presence of MT1-MMP in benign epithelial cells (near cancerous tissue) from these patients might in fact be induced by the cancer. A study by Paterson *et al.*^[18] in bladder cancer was cross-referenced, which had further suggested that the so-called “benign” epithelial cells in those patients were in fact not benign at all, but genotypically abnormal. These findings have been encountered by others and are worthy of further study.

Discrepancies between gene expression (higher MT1-MMP expression in benign prostate hyperplasia and prostate cancer tissues, when compared to normal prostate) and protein expression (lower expression in prostate cancer tissues compared to normal prostate and benign prostate hyperplasia) were noted by Neuhaus *et al.*^[19]. These results contrast with most other published studies, which led the authors to speculate that cells of the prostate interstitium (included in assessment of total immunofluorescence due to MT1-MMP) may have increased protein expression and may account for the apparent levels of protein expression seen in epithelial cells.

Cardillo *et al.*^[20] analysed 38 paraffin-embedded samples from prostate cancer patients (who had undergone radical prostatectomy) by

immunohistochemistry and compared prostate intraepithelial neoplasia (PIN) and its normal adjacent prostate (NAP) counterpart. MT1-MMP was observed to be more strongly expressed in tumor tissue than in PIN and NAP tissue, with the expression of MT1-MMP reaching its highest levels in the most aggressive prostate tumors with high Gleason scores (Gleason scores are used to grade prostate cancer, with a score above 7 indicative of aggressive, metastatic disease). Once again, expression was detected in surrounding stroma and epithelia, backing up the findings of Trudel *et al.*^[17]. The authors speculate that stromal and tumor cells could co-operate in facilitating tumor cell invasion, hence the requirement for MT1-MMP expression, and that transition from benign epithelium via PIN to cancer is associated with changes in localisation of MT1-MMP in the prostate epithelium^[20].

In a further 40 patients, Reis *et al.*^[21] also monitored tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) expression together with that of MT1-MMP. TIMPs inhibit some MMPs and other protease enzymes, but not MT1-MMP^[22]. The loss of TIMP-1 protein expression was correlated to cancer progression, with MT1-MMP protein expression being identified in the majority of prostate cancer specimens. Once again, a positive correlation with Gleason score was observed^[21]. This reinforced an earlier study by the same group (79 prostate cancer patients), which indicated that MT1-MMP expression was higher in patient samples with Gleason scores ≥ 7 , though that dataset only exhibited marginal statistical significance^[23].

Upadhyay *et al.*^[24] investigated the relationship between the MT1-MMP and MMP-2 expression, with immunohistochemistry confirmed by western blotting and gelatin zymography. A significant correlation between the pattern of MMP-2 and MT1-MMP expression within the epithelial components of individual specimens was observed. Differential staining was seen between benign epithelia, high-grade PIN, and prostate cancer. In benign glands, the greatest expression for MT1-MMP was in basal cells (BCs), whereas secretory cells were rarely positive. Conversely in high-grade PIN, secretory cells showed consistent cytoplasmic staining. In cancer cells, staining was heterogeneous and varied from no staining to very intense staining in select glands^[24].

High expression of insulin-like growth factor-1 receptor (IGF-1R) in prostate cancer was identified by Sroka *et al.*^[25], who suggested that using MT1-MMP localization and IGF-1R expression may serve as a predictive biomarker of aggressive disease. MT1-MMP expression was high in the apical regions of the

luminal cells in PIN and prostate cancer cells, though less intense in the basolateral regions of benign tissues. IGF-1R was expressed primarily in the basal cells of normal glands and highly expressed in prostate cancer tissues^[25].

As is the case with studies on cell lines, the majority of clinical reports focus on expression of MT1-MMP. Both Jung *et al.*^[11] and Riddick *et al.*^[26] compared a wider range of MT-MMP family member gene expression in paired tissue samples from non-malignant and malignant parts of the same prostate cancer patient biopsies, using real-time polymerase chain reaction (PCR). Interestingly, Jung *et al.*^[11] identified a significant down-regulation for all investigated MT-MMPs except for MT2-MMP in malignant tissue and did not detect a correlation between tumor classification and MT-MMP expression. Riddick *et al.*^[26] investigated *MT-MMP* gene expression in 44 prostate cancer cases and 23 benign prostate hyperplasia specimens, also by real-time PCR. This study additionally found increased gene expression of MT2-MMP, MT5-MMP and MT6-MMP in malignant tissue compared to benign prostate tissue, and suggested that these proteases are likely to participate directly in prostate tumor invasion. It is important to note that MT6-MMP was primarily expressed by the epithelial cancer cells rather than stromal cells. The lack of difference in MT1-MMP expression between malignant and local non-malignant tissue perhaps provides further evidence for the conclusions previously discussed^[17,18,20].

The evidence for MT-MMP expression, and particularly MT1-MMP expression, in prostate cancer cells and tumors from patients is compelling. The picture is complicated by the involvement of the tumor microenvironment, in which MT-MMP expression is influenced, perhaps initiated, by the development of cancer. In the following section, the roles of individual MMPs are considered, along with links to other pathways known to be important in prostate cancer progression and metastasis.

FUNCTIONS OF MT-MMPs IN PROSTATE CANCER PROGRESSION AND METASTASIS

It is widely accepted that MT-MMPs play key roles in the metastatic process^[27]. With regards prostate cancer, MT-MMPs have been identified as contributing towards apoptosis, angiogenesis, proliferation and metastasis^[7]. MT-MMPs have been shown to be involved in various molecular processes in prostate cancer progression and metastasis, which will now be considered here, together with information regarding

potential regulatory pathways. Some of the molecular events associated with MT-MMP expression and function in tumor cell migration, angiogenesis and vascular signalling are summarised in Figure 1^[28].

MT1-MMP plays a role in epithelial-to-mesenchymal transition (EMT). EMT is an important process in the metastatic cascade, involving multiple oncogenic drivers^[29]. Cao *et al.*^[30] initially used DNA microarray database mining to reveal upregulation of MT1-MMP in human primary and metastatic prostate cancer samples. Using 3D cell culture models, the study additionally demonstrated that transformation of LNCaP cells with MT1-MMP induced morphological changes and modulation of epithelial and mesenchymal markers consistent with EMT, and thus metastatic transformation. Further experiments demonstrated that these MT1-MMP-induced phenotypic changes were linked to Wnt5a, also associated with EMT^[31]. These findings are supported by the aforementioned study by Jennbacken *et al.*^[14], which similarly described E-cadherin downregulation and N-cadherin upregulation (both consistent with EMT) in the androgen-independent LNCaP-19 cell line, accompanied by increased MT1-MMP.

Degradation of the extracellular matrix (ECM), and specifically laminin-10 (Ln-10), was explored by Bair *et al.*^[32]. Laminins are key glycoprotein components of the ECM: providing structural support to the basal lamina in both normal prostate and malignant tissue. The authors point to evidence previously published describing upregulation of Ln-10 as prostate cancer progresses from normal to PIN through to invasive cancer, suggesting a role for MT1-MMP in the invasion of prostate cancer^[33]. Here, recombinant MT1-MMP (and MT1-MMP-expressing cells and tissues) was shown to cleave the $\alpha 5$ chain of purified human Ln-10 from its 350-kDa form into specific fragments. This cleavage was shown to decrease cell adhesion to purified Ln-10, and to increase transmigration of DU-145 cells through cleaved Ln-10 and thus the basal lamina. Increased invasion mediated by MT1-MMP was also observed by Wang *et al.*^[34]. Using cells engineered to overexpress MT1-MMP (namely PC3-LN4), invasion into type-I collagen gels *in vitro* was observed, through activation of pro-MMP2. PC3-LN4 cells additionally proliferated at a faster rate than mock-transfected control cells when grown subcutaneously in nude mice.

Endo180 (uPARAP, urokinase-type plasminogen activator receptor-associated protein) regulates collagen remodelling and chemotactic cell migration through cooperation with MT1-MMP. A study by Kogianni *et al.*^[35] describes how Endo180 is

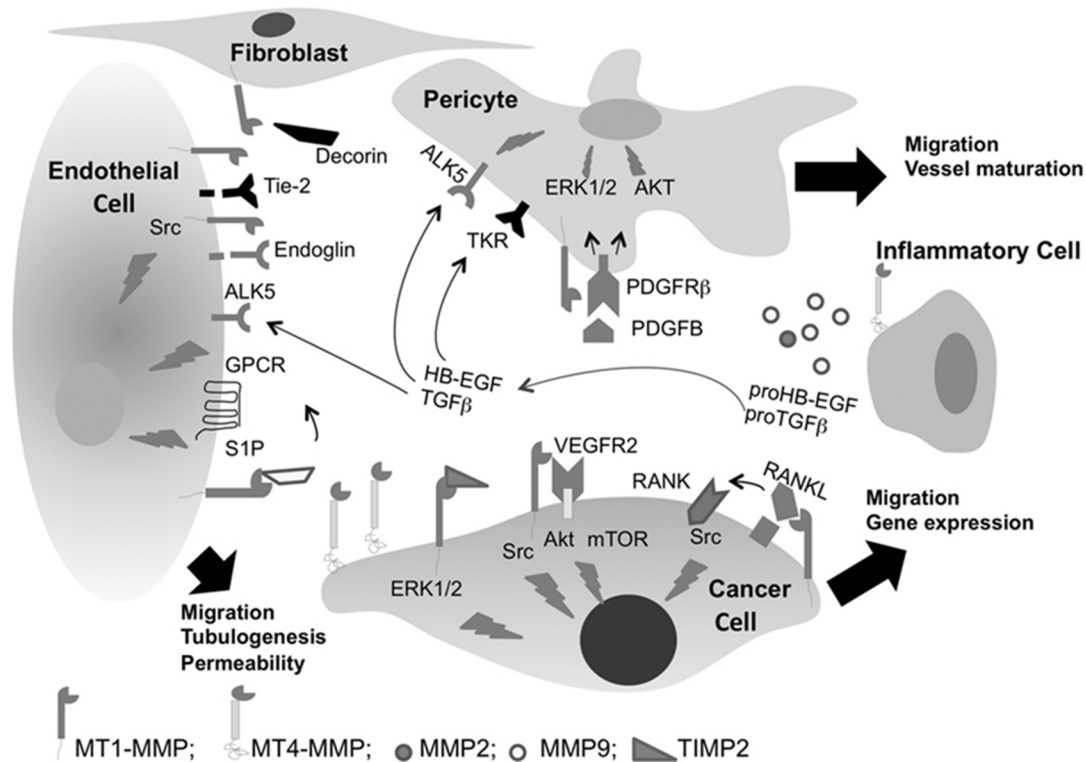


Figure 1: Membrane type-MMPs regulate cell signalling in cancer. MT1-MMP regulates cell migration through both ECM proteolysis and non-proteolytic-dependent TIMP-2 activation of ERK1/2 pathway. MT1-MMP regulates VEGF gene expression through Src, Akt, and mTOR activation and stimulates tumor angiogenesis. Roles for MT-MMP expression are also associated with TGF β , Tie-2 and PDGFR β . Figure was originally published by Sounni *et al.*^[28]. MT-MMP: membrane-type matrix metalloproteinases; ECM: extracellular matrix; VEGF: vascular endothelial growth factor

positively correlated with prostate cancer clinical risk and suggests that it is a stronger predictor of Gleason score than serum PSA. Endo180 disrupts epithelial cell contact and plays a potential role in EMT in prostate cancer. Endo180 and MT1-MMP co-expression was identified as strongly upregulated in the stroma of prostate cancer with low clinical risk, indicating that tumor-associated stromal fibroblasts can acquire the ability for effective collagen degradation and internalisation at the early stages of tumor development. These findings are interesting and add further support to prostate cancer cells influencing MT1-MMP expression in their surrounding microenvironment.

The transcription factor p53 is known to play significant roles as a tumor suppressor in cancer progression. Wang *et al.*^[36] considered the involvement of p53 in prostate cancer cell invasion and metastasis, using DU145 cells in which p53 was silenced by siRNA. Increased invasion and metastasis were observed in a series of *in vitro* experiments, including increased MT1-MMP expression and activity, along with that of MMP-2 and MMP-9. These findings are also consistent with the studies associating MT1-MMP with EMT, as demonstrated by reduced E-cadherin,

increased N-cadherin and enhanced vimentin staining. The authors additionally provide evidence that these effects are mediated via FAK-Src signalling.

A study by Sankpal *et al.*^[37] provides evidence for regulation of MT1-MMP by specificity protein 1 (Sp1). Sp1 is expressed in a number of different cancers, and plays key regulatory roles in processes associated with prostate cancer progression and metastasis. DU-145 cells were reported to express constitutively phosphorylated ERK, while PC3 and PC3N cells express constitutively phosphorylated AKT/PKB and c-Jun NH2 terminal kinase (JNK). Interestingly, both MT1-MMP and Sp1 levels were decreased in PC3 cells when PI3K and JNK were inhibited, and MT1-MMP levels were decreased in DU-145 cells when MEK was inhibited. These results suggest Sp1-mediated transcriptional regulation of MT1-MMP in prostate cancer cell lines via differential signalling control^[13,37]. Sroka *et al.*^[25] additionally considered the relationship between IGF-1R and MT1-MMP in prostate cancer cells and tissues, the expression data for which were discussed earlier. IGF-1R has been identified to play a role in prostate cancer metastatic progression, through PI3K, MAP kinase and ERK signalling^[38]. Interestingly, decreased MT1-MMP expression at mRNA and

protein level resulted from inhibition of IGF-1R (using picropodophyllin) in PC3N cells. Increased IGF-1R, when activated by IGF-1, led to increased MT1-MMP expression and activity following treatment of LNCaP cells with synthetic androgen R1881.

Reversion-inducing cysteine-rich protein with Kazal motifs (RECK), originally found to suppress transformation caused by the oncogene KRAS, is a glycoprotein tumor suppressor which inhibits metastasis and angiogenesis^[39]. Previous studies have identified RECK as an inhibitor of various MMPs, including MT1-MMP^[40]. Rabien *et al.*^[41] identified RECK expression in prostate cancer cell lines and tissue and observed a significant decrease in malignant tissue. Significantly, RECK overexpression led to a dramatic reduction in tumor cell invasion and a decrease of pro-/active MT1-MMP expression (up to 53% of control).

Filiz and Dass^[42] demonstrated decreased expression of MT1-MMP associated with reduced pigment epithelium-derived factor (PEDF). The authors noted that PEDF had been previously found to be downregulated in prostate cancer patients (specifically in high-grade PIN, the most likely precursor of prostate cancer)^[43]. PEDF was examined for effects on PC3 cells, with increased adhesion to ECM protein collagen-I and decreased expression of phosphorylated FAK observed. Tumor cell invasion through collagen-I was also reduced. These findings were attributed to the decreased expression of MT1-MMP.

Increased expression of both MT1-MMP and LIM kinase 1 (LIMK1) in prostate tumor tissues was reported by Tapia *et al.*^[44]. LIMK1 is a downstream effector of Rho signalling, modulates actin dynamics and is overexpressed in prostate cancer cells, where it promotes invasion and metastasis. Results showed that treatment with ilomastat (broad-spectrum hydroxamate-based MMP inhibitor) reduced LIMK1-induced invasion of benign prostate epithelial cells (BPH-1 cells) suggesting that the process is mediated by MMPs, notably MT1-MMP. Increased MT1-MMP expression in cells overexpressing LIMK1 was also reported, along with transcriptional activation and localisation of protein to the plasma membrane. LIMK1 was shown to physically associate with MT1-MMP and to co-localise with it in Golgi vesicles, thereby enabling transport of MT1-MMP to the cell surface^[44].

FGFR4 expression and polymorphism has been linked to prostate cancer progression and drug resistance^[45]. In particular, a single nucleotide polymorphism (SNP)

in codon 388 of the human *FGFR4* gene has been linked to poor prognosis in prostate cancer patients. This SNP results in Gly388 being transformed to Arg in the transmembrane domain of the receptor, leading to prolonged FGFR4 receptor activation^[46]. MT1-MMP and FGFR4 were found to be co-expressed in the tumor edges and prostate carcinoma: MT1-MMP upregulation was observed in cancer cells (9 of 14) and/or reactive stroma (9 of 14), whereas FGFR4 expression was mainly found in the tumor cells. FGFR4-R388 was shown to enhance MT1-MMP-mediated prostate cancer cell invasion. FGFR4 was thus also identified as playing a role in MT1-MMP-dependent ECM degradation and tumor progression involving EMT *in vivo*^[47].

MT1-MMP has additionally been associated with oxidative stress in prostate cancer cell lines. Nguyen *et al.*^[48] described how expression of MT1-MMP increased oxidative DNA damage via reactive oxygen species (ROS) in LNCaP and in DU145 cells, causing oxidative stress. The study confirmed the findings of others in demonstrating that MT1-MMP is associated with a more aggressive phenotype as illustrated by increased cell migration, invasion and anchorage-independent cell growth. Use of the scavenger *N*-acetylcysteine to block ROS activity inhibited the MT1-MMP-mediated increase in cell migration and invasion. The authors additionally suggested a role for β 1-integrins in facilitating cell adhesion to matrix proteins, and that this was necessary for induction of ROS in MT1-MMP-expressing prostate cancer cells.

PTEN (phosphatase and tensin homologue deleted on chromosome ten) is a phosphatase enzyme involved in regulation of PI3K-Akt pathway signalling and thus cancer progression. Most metastatic prostate cancers exhibit loss-of-function mutations or deletions of this key tumor suppressor^[49]. Kim *et al.*^[50] considered the role of PTEN inactivation on MT-MMP expression in prostate cancer. Mouse PTEN null cells exhibited up-regulation of MT1-MMP and MT3-MMP gene expression (and the associated increased migration and invasion), and increased MT1-MMP protein expression *in vivo*. Interestingly, the MT1-MMP displayed by PTEN null cells exhibited a slow rate of turnover, which was thought to be due to differential O-glycosylation of the MT1-MMP hinge region modulating enzyme stability. MT1-MMP expression in PTEN null cells was additionally determined to be regulated by PI3K/Akt but not MAPK signalling, as determined by inhibitors of those pathways. A role for downstream pathway mTORC1 (positively regulates translation thereby promoting protein synthesis^[51]) was predicted, given the upregulation of MT-MMP protein

expression, but intriguingly inhibition by rapamycin (an mTOR inhibitor) actually upregulated MT1-MMP protein expression in PTEN null cells further, an effect that was reversed by Akt inhibition. The authors discuss this potential side effect of rapamycin, and note that similar observations have been reported elsewhere^[50].

As was the case with expression data, information regarding the roles of the other members of the MT-MMP family is somewhat limited. Delassus *et al.*^[12] identified a series of prostate cancer progression modulators and engineered overexpression in prostate cancer cells (PC3) and others. Changes in gene expression of MT1-, MT3- and MT6-MMP were then evaluated. Over-expression of activator protein-2 α , interleukin 4 and p16^{INK4a} had no effect on MT-MMP expression. Fibulin1D led to down-regulation of MT1- and MT3-MMP (no reliable data for MT6-MMP). Supporting the work of Wang *et al.*^[36], p53 over-expression led to reduced MT1-MMP expression (no reliable data for the other MT-MMPs evaluated). Over-expression of PTEN produced no change in MT1-MMP expression, which is at odds with the data of Kim *et al.*^[50], with the caveat that cells in that study were mouse prostate cancer cells. Upregulation of MT3-MMP was observed, however. Furthermore, raf kinase inhibitor protein over-expression led to increased MT1-MMP, with no change detected for the others. Finally, over-expression of E-cadherin led to reduced expression of all three MT-MMPs. This finding thus reinforces the observations discussed earlier regarding the role of MT-MMPs in EMT (i.e. a phenotypic shift from E- to N-cadherin expression)^[14,30,36].

Lin *et al.*^[52] undertook genomic association studies to identify genetic variants, i.e. SNPs utilising data from the Cancer Genetic Markers of Susceptibility dataset, which includes data from 1,151 prostate cancer patients. The authors explain how SNP-SNP interactions, rather than studying individual SNPs, potentially have greater impact on unravelling the underlying mechanisms of complex disease^[52]. Three important SNP-SNP interactions were identified, linking MT3-MMP to ROBO1, CSF-1 and EGFR. ROBO1 is a member of the roundabout immunoglobulin superfamily, and has been identified as playing key roles in prostate cancer progression^[53]. It is cleaved by MMPs and translocates into the nucleus of cancer cells, which perhaps suggests a signalling role. The authors suggest that as no specific MMP has to-date been linked to ROBO1 cleavage, these data may have uncovered the potential for such a role for MT3-MMP. Colony stimulating factor-1 has been associated with increased tumor angiogenesis^[52]. Epidermal growth

factor receptor (EGFR) plays an important role in regulating cancer cell growth and function, not least in prostate cancer, and is therapeutically important^[54]. More recently, a much wider study by the same group using the prostate cancer PRACTICAL consortium data with approximately 21,000 patients, identified four key SNP-SNP interactions found to be associated with prostate cancer aggressiveness. Of relevance here, this study again linked MT3-MMP and EGFR^[55].

In addition, several studies point to an important role for MT-MMPs, and MT1-MMP in particular, in the processes associated with metastatic spread to bone in prostate cancer. This data will be considered in the following section.

EXPRESSION AND ROLES OF MT-MMPs IN PROSTATE CANCER BONE METASTASIS

It is suggested that more than 80% of patients with disseminated prostate cancer will present with metastasis to the bone^[56,57]. Skeletal complications are thus one of the leading causes of morbidity and mortality in prostate cancer patients. The normal equilibrium between osteoblastic and osteolytic activity in bone is disturbed in prostate cancer, leading to changes that are likely to provide a favourable microenvironment for metastatic colonisation. Given the established roles for MMPs in normal bone remodelling, a role for MMPs in prostate cancer bone metastasis has long been proposed^[58]. With this in mind, it is perhaps surprising that relatively little research has been directed to the MT-MMPs in this area to-date. MT1-MMP knock-out mice exhibit severe skeletal abnormalities, confirming a role in normal bone maintenance and development^[59]. Given these roles in normal bone health, the expression of MT-MMPs in prostate cancer cells and tissues led Bonfil *et al.*^[58] to speculate as to the existence of a selective process in which prostate cancer cells may have a greater propensity to metastasise to bone, or whether the microenvironment within the bone itself may induce MMP expression in prostate cancer cells, after their arrival at the bone.

Nemeth *et al.*^[60] evaluated MT-MMP expression in clinical samples and the role of MMP activity in prostate cancer that had metastasised to the bone, using a preclinical mouse model of bone metastasis employing PC3 xenografts. MT1-MMP protein expression (as identified by immunohistochemical staining) was consistently observed in the 18 core bone biopsy samples from prostate cancer patients. In preclinical studies, single human foetal bone fragments were implanted subcutaneously in immunodeficient

mice. After an interval of 4 weeks, PC3 cells were then injected directly into some of the implants, with/without initiation of daily treatment with broad-spectrum MMP inhibitor batimastat for 2 weeks. MT1-MMP expression was subsequently identified in PC3 bone tumors, localised primarily to tumor cells, with some stromal expression noted. The PC3 bone tumors were mostly osteolytic in nature, and MMP inhibition by batimastat reduced the number of osteoclasts per millimetre in these implants. The authors concluded that MT1-MMP activity in prostate cancer cells appears to be crucial in bone matrix turnover. This, together with metastatic tumor growth, appeared to be linked in cycle that is disrupted by MMP inhibition^[60].

A further correlation between MT1-MMP expression in prostate cancer cells and bone metastasis was also reported by Bonfil *et al.*^[61]. MT1-MMP expression was abundant and consistent in tumor cells identified in paraffin sections of bone metastases from 20 prostate cancer patients (androgen independent disease). It should be noted that MT1-MMP expression was noted in endothelial cells, osteocytes, osteoblasts and stroma in matched normal bone samples, consistent with a role in bone development, albeit at expression levels which appear significantly lower than those exhibited by the tumor cells. Preclinical models were utilised to examine the role of MT1-MMP in metastatic bone colonisation of prostate cancer cells. MT1-MMP was introduced into LNCaP cells, while it was silenced (using siRNA for MT1-MMP) in DU145 cells. MT1-MMP over-expression enhanced bone tumor growth (via intra-tibial injection) and associated osteolysis, while not affecting cell proliferation *in vitro* or subcutaneous tumor growth *in vivo*. This led the authors to conclude that MT1-MMP contributes a unique stimulatory effect on tumor growth in the bone microenvironment. Further studies utilising orthotopic models which better replicate the normal disease dissemination process are required to confirm these findings, but the authors nevertheless suggest the possibility that MT1-MMP activity may be worthy of pursuing as a therapeutic target for prostate cancer bone metastases. Furthermore, a role for RANKL was suggested: RANKL (receptor activator of NF- κ B ligand) is a regulator of osteoclastogenesis, and its release in the bone microenvironment was linked to MT1-MMP activity. siRNA knockdown of MT1-MMP inhibited bone tumor growth of DU145 cells and simultaneously led to osteogenesis, a phenomenon for which mechanistic information was not obtained. It was suggested that MT1-MMP inhibition may have shifted the balance toward bone formation simply by inhibition of osteolysis/osteoclastogenesis^[61].

Sabbota *et al.*^[62] subsequently followed up their initial findings and provided further evidence for a link between RANKL shedding and MT1-MMP protein expression, summarised in Figure 2. In this study, conditioned media from LNCaP cells expressing both RANKL and MT1-MMP was shown to enhance cell migration of LNCaP-C4-2b cells, which are MT1-MMP deficient. This was inhibited by osteoprotegerin (soluble decoy receptor of RANKL) and selective MT1-MMP inhibitor MIK-G2. The authors hypothesised that these findings indicated that MT1-MMP enhances tumor cell migration through initiation of an autocrine loop requiring RANKL shedding in prostate cancer cells. Evidence was also provided for a role for Src as a downstream mediator of RANKL^[62].

The importance of cadherin-11 in prostate cancer bone metastasis was considered by Huang *et al.*^[63]. Cadherin-11 is an osteoblast cadherin, identified as being aberrantly expressed in prostate cancer cells derived from bone metastases^[64]. LNCaP-C4-2B4 cells in which expression of cadherin-11 had been engineered demonstrated increased spread and intercalation into an osteoblast layer *in vitro* and exhibited enhanced migration and invasion. Downregulation of cadherin-11 in PC3 cells, which naturally express cadherin-11, decreased cell migration and invasion. A possible role for MT2-MMP was suggested, following gene array analysis of the LNCaP-C4-2B4 cells. Several genes related to invasion and metastasis were identified as upregulated, among which MT2-MMP was a prominent finding^[63]. Interestingly, *IGF-1* gene expression was also reported as upregulated, which supports the evidence provided by Sroka *et al.*^[25] relating to a role for IGF-1R in MT-MMP expression and activity, as discussed earlier.

MT-MMPs clearly play important roles in the development of metastatic bone deposits. Given that most patients with prostate cancer ultimately succumb to metastasis, a strategy to specifically target MT1-MMP-expression on tumor cells may prove an attractive means by which to address prostate cancer metastasis to bone.

OPPORTUNITIES FOR DRUG TARGETING

The expression of MT-MMPs in prostate cancer and associated bone metastases suggests an opportunity for targeted therapy. As an example, the Bonfil group focused on MT1-MMP expression in prostate cancer bone metastases, and suggested that the increased MT1-MMP activity was worthy of pursuing as a therapeutic strategy. The group later

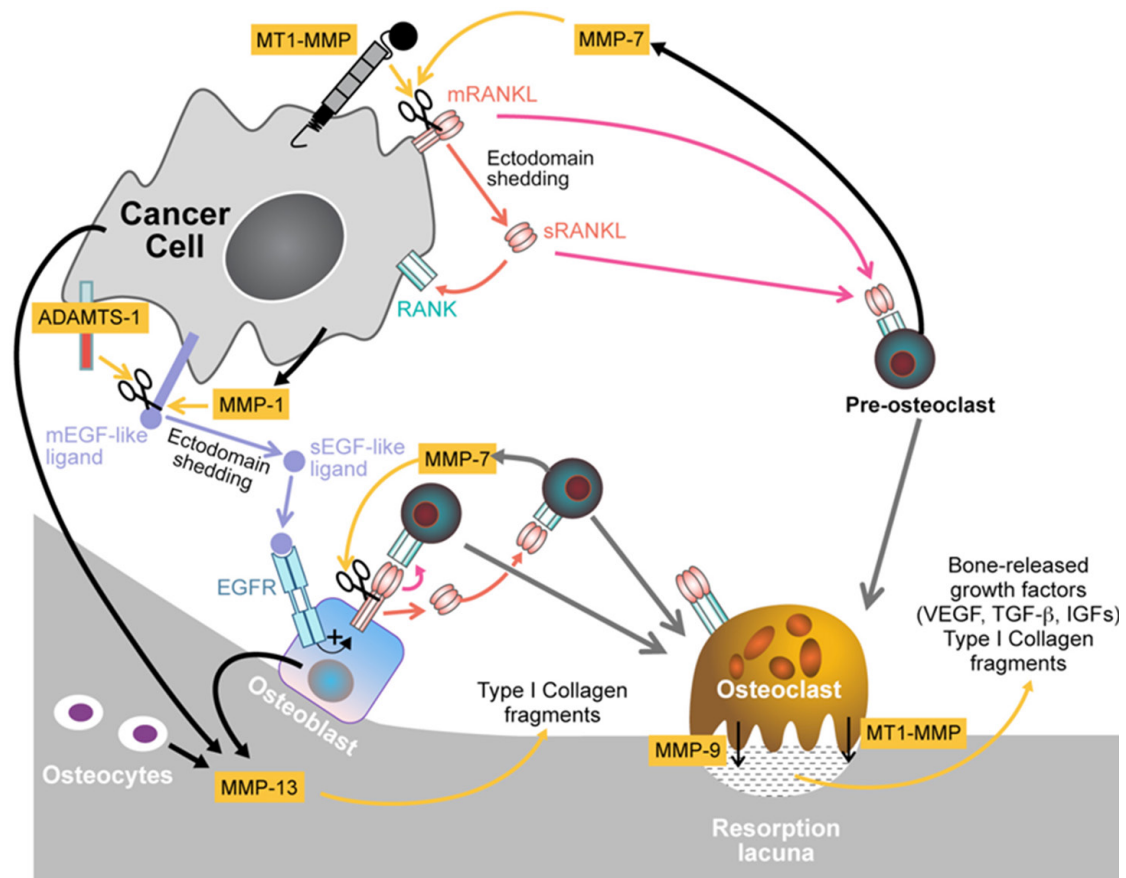


Figure 2: Diagram representing the most representative roles of MT1-MMP at bone metastatic sites. MT1-MMP expression by cancer cells results in proteolytic cleavage of tumor-associated membrane-bound RANKL (mRANKL), generating a soluble form of RANKL (sRANKL) that activates RANK favouring migration of tumor cells and osteoclastogenesis, respectively. Roles for MMP-7, MMP-9 and MMP-13 were also described. Reprinted by permission from Macmillan Publishers Ltd.^[78]. MT-MMP: membrane-type metalloproteinases

went on to propose a combination of Src inhibition with RANKL and/or selective MT1-MMP inhibition as a strategy, particularly for prostate cancer patients with bone metastases. Other strategies are worthy of investigation, utilising the knowledge that has been gained in understanding key pathways and how they interact with MT1-MMP activity, as have been discussed. Further study is required to fully understand the expression and roles of MT-MMPs in normal cells in the tumor microenvironment, however, given the possibility that tumor cells may induce expression in benign epithelial cells. Furthermore, very few studies contain data from healthy individuals for true “normal” tissue comparison.

MMP inhibition is an area that has been well explored in the past by big pharma, but has yet to fulfil its clinical potential. This is due to the complex roles of individual MMPs and their inter-connected compensatory mechanisms, poor clinical trial design, and drugs lacking exquisite selectivity^[2,65]. Utilising MMP expression and proteolytic activity, however,

is perhaps a more attractive approach^[5,66]. This is especially true given the role of MMPs in angiogenesis and in maintenance of tumor vasculature^[67,68]. While not specifically studied in prostate cancer, evidence for other cancers is compelling, particularly for MT1-MMP^[66,67].

The need for novel therapeutics in metastatic prostate cancer is clear. The burden of drug toxicity endured by many patients, often elderly and frail, means that an emphasis must be placed on targeted agents with minimal side effects. Small molecule chemotherapeutics remain central to prostate cancer therapy, but despite some considerable recent advances, these new agents still suffer from a lack of selectivity and dose-limiting toxicities. There is therefore considerable interest in the development of prodrugs to tailor the pharmacokinetics of molecules in favour of tumor-selective drug targeting, thereby decreasing these dose-limiting side effects and enhancing therapeutic index^[69]. Multiple reviews have been published covering a huge range of approaches for cancer drug delivery, but it is fair to say that

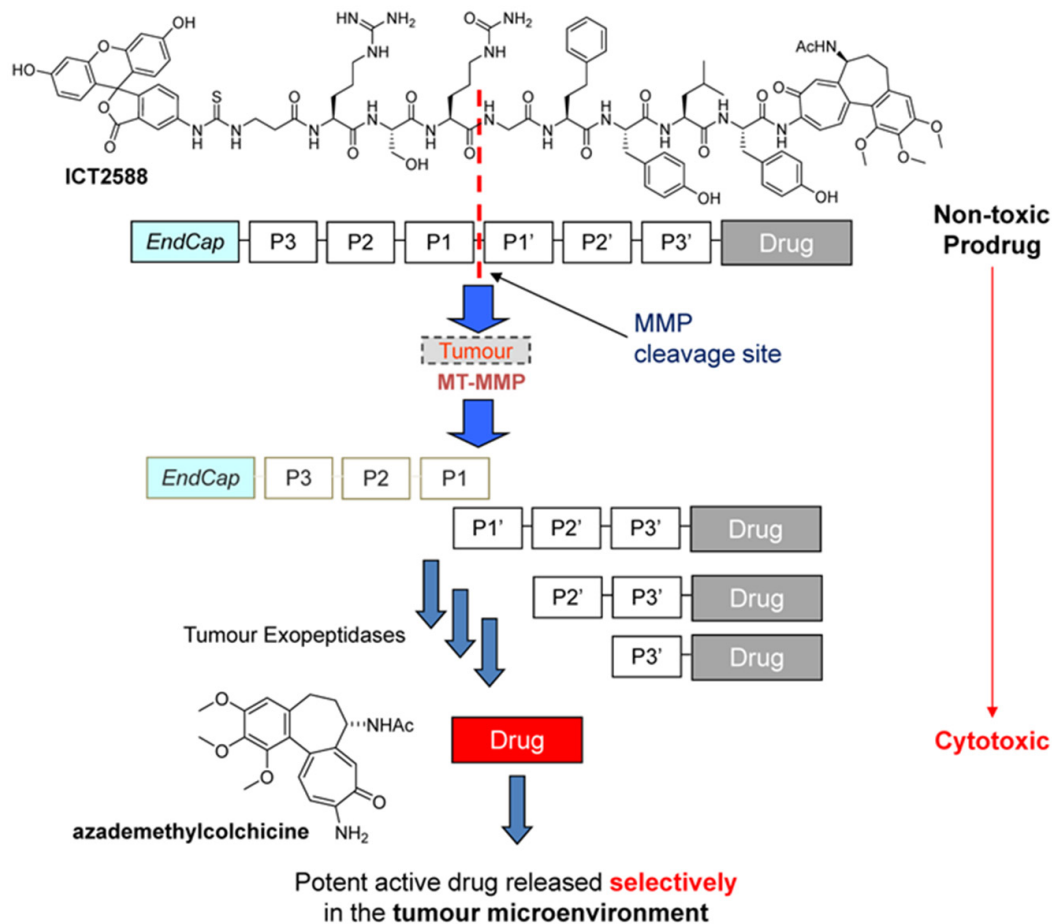


Figure 3: Structure of ICT2588 and MT-MMP targeting concept. Peptide conjugation renders the drug (azademethylcolchicine) pharmacologically inactive. Following initial MT-MMP recognition and cleavage, the remaining amino acids are metabolised to release the toxic drug selectively in the tumor microenvironment. MT-MMP: membrane-type matrix metalloproteinases

prodrug approaches specifically developed for prostate cancer are limited. MMP-activated prodrugs in prostate cancer are considered by Barve *et al.*^[70]. Here the concept of attaching an MMP-recognition peptide to a drug is discussed. Peptide conjugation renders the drug inactive (thereby creating the prodrug) until such time that tumor-expressed MMPs recognise and cleave the peptide to release the drug. This area has considerable potential. Further examples are provided by Choi *et al.*^[3] and Law and Tung^[4].

ICT-2588 is a prodrug of azademethylcolchicine (an analogue of colchicine^[71]) and is a potent anti-vascular agent [Figure 3]. Activated by MT1-MMP, ICT-2588 has shown promise in preclinical studies, successfully achieving enhanced therapeutic index^[72], an absence of cardiotoxicity^[73], and activity in a range of tumor types, not least prostate cancer (activity in PC3 xenografts in mouse models)^[73]. This potential for the treatment of prostate tumors led the authors to apply the same technology to paclitaxel, yielding ICT-3205^[74]. This agent provides for enhanced

tumor delivery of paclitaxel in preclinical studies (PC3 xenograft in mice), realising 10-fold increases in tumor concentrations (as measured by *in vivo* pharmacokinetics studies) while decreasing the exposure of drug to normal tissues, and associated toxicities. Given the findings of the STAMPEDE prostate cancer trial supporting earlier use of taxanes in treatment of metastatic prostate cancer^[75-77], this approach is particularly timely.

While our understanding of the roles of MT-MMPs in prostate cancer and metastatic disease is growing all the time, much is still to be learnt. The potential for exploiting the proteolytic capacity of these enzymes is without question, but it remains to be seen whether a clinically useful drug molecule will emerge.

DECLARATIONS

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Authors' contributions

R.A. Falconer and P.M. Loadman contributed equally to writing this review.

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Conflicts of interest

RAF and PML declare that they are founding shareholders of Incanthera Ltd.

Patient consent

Not applicable.

Ethics approval

Not applicable.

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