OPEN ACCESS

GAVIN PUBLISHERS

Journal of Oncology Research and Therapy

Satyam LK, et al. J Oncol Res Ther: JONT-145.

DOI: 10.29011/2574-710X. 000045

Research Article

A Selective Matriptase Inhibitor Blocks Tumor Growth and Metastasis While Displaying Potent Antiangiogenic Activity in an Orthotopic Murine Renal Cell Cancer Model

Leena K. Satyam[1], Rajeev Goswami[1], Chakshusmathi Ghadiyaram[1], Anu Moilanen[2], Tarja Ikonen[3], Gerd Wohlfahrt[3], Jwala Nagaraj[1], Krishnaprasad Subbarao[1], Raghuveer Ramachandra[1], Hosahalli S. Subramanya[1], Pekka Kallio[2], Murali Ramachandra[1*]

[1]Aurigene Discovery Technologies Limited, Bangalore, India

[2]Orion Corporation, Tengströminkatu 8, Turku, Finland

[3]Orion Corporation, Orionintie 1, Espoo, Finland

[*]**Corresponding author:** Murali Ramachandra, Aurigene Discovery Technologies Limited, 39-40, KIADB Industrial Area, Electronic City, Phase II, Hosur Road, Bangalore-560034, India. Tel: +91-8071025444; Fax: +91 80 28526285; Email: murali_r@aurigene.com

Citation: Satyam LK, Goswami R, Ghadiyaram C, Moilanen A, Ikonen T, et al. (2018) A Selective Matriptase Inhibitor Blocks Tumor Growth and Metastasis While Displaying Potent Antiangiogenic Activity in an Orthotopic Murine Renal Cell Cancer Model. J Oncol Res Ther: JONT-145. DOI: 10.29011/2574-710X. 000045

Received Date: 09 February, 2018; **Accepted Date:** 07 March, 2018; **Published Date:** 15 March, 2018

Abstract

Matriptase, a type II transmembrane serine protease, is expressed by cells of surface epithelial origin and involved in the progression of various cancers. Previously, significant immune histochemical expression of matriptase in subtypes of RCC with no expression in areas of RCC with sarcomatous differentiation and normal collecting tubules was reported. In this study, we tested if matriptase inhibition can impact primary tumor growth and invasive potential in a model of renal cancer. Initially, the impact of selective matriptase inhibitor compound-15 was evaluated on a murine renal carcinoma cell line *in vitro*. Results indicated a dose-dependent inhibition of Renca cell proliferation only when cells were cultured in a matrix-independent manner as well as in migration and invasion. In the orthotopic Renca kidney cancer model, compound-15 treatment resulted in dose-dependent inhibition of primary tumor growth and metastasis, which correlated well with inhibition of angiogenesis. An additive effect on tumor growth and metastasis was observed when compound-15 at 0.5 mg/kg was combined with sorafenib, a kinase inhibitor, at 10 mg/kg. Preclinical evidence presented here provides a rationale for inhibition of matriptase with or without sorafenib as a therapeutic modality in renal cancer.

Keywords: Angiogenesis; Invasion; Matriptase; Migration; Protease; Renal Cell Carcinoma

Introduction

Matriptase, referred to as MT-SP1 (membrane type serine protease type 1), prostamin, TADG-15, PRSS14, SNC19, and ST14, is a type II transmembrane serine protease often found in complex with its cognate inhibitor, Hepatocyte Growth Factor Activator Inhibitor-1 (HAI-1) [1,2]. Matriptase is broadly expressed by epithelial and carcinoma cells and has been shown to be over-expressed in many tumor types [3,4], most importantly in prostate adenocarcinoma [5,6].

In epithelial cells matriptase carries out essential functions in development, differentiation, and maintenance of epithelial barrier homeostasis. Matriptase knockout mice die shortly after birth due to severe dehydration caused by impaired epidermal barrier function, indicative of a critical role in development [7]. The oncogenic activity of matriptase derives from its ability to trigger pro-oncogenic and pro-metastatic substrates in the cell or linked to cell membrane such as Hepatocyte Growth Factor (HGF), Protease-Activated Receptor-2 (PAR-2), Urokinase-type Plasminogen Activator (uPA), platelet-derived growth factor-D from their corresponding pro-growth factor forms [8-10].

Activation of uPA by matriptase plays a vital role in angiogenesis, tumor invasion and metastasis [11]. Matriptase can exhibit potent oncogenic activity via Ras-dependent and Ras-independent pathways even when it is slightly overexpressed in the skin of transgenic mice [12]. Matriptase is known to activate cell growth as well as angiogenesis [13]. Angiogenic potential of secreted matriptase has also been described in cancer cells especially under hypoxic conditions [14]. Matriptase has been found to activate stromelysin (MMP-3), which may promote tumor growth and angiogenesis [15]. CVS-3983, a potent and selective small molecule matriptase inhibitor has been found to have an inhibitory effect on the invasive capacity of androgen-independent prostate cancer cells *in vitro* and growth of the androgen-independent human prostate cancer xenograft models in vivo [16]. Matriptase is therefore considered as an attractive target for cancer therapy.

Renal Cell Carcinoma (RCC) is the fourth most common genitourinary cancer with poor prognosis and limited treatment options in advanced stage. Angiogenesis is a marked feature in clear cell RCC, the most common type of RCC, which accounts for approximately 70% to 80% of cases [17]. Angiogenesis inhibitors have shown clinical benefit in patients with advanced renal cell cancer, but further therapeutic options are needed. All subtypes of RCC showed significant immune histochemical expression of matriptase [18]. In contrast, no expression occurred in areas of RCC with sarcomatous differentiation (SRCC) or in normal collecting tubules.

In this study, we aimed to find out if matriptase inhibition can reduce primary tumor growth and metastasis in a model of renal cancer. Our findings demonstrate the efficacy of a recently described potent and selective small molecule matriptase inhibitor [19] with a potent anti-angiogenic activity in tumor growth inhibition and metastasis in an aggressive orthotopic renal cancer model. To the best of our knowledge, this is the first report of potent anti-angiogenic potential of a matriptase inhibitor in a renal tumor model.

Materials and Methods

Primary Screening Assay and Selectivity Assays

The recombinant matriptase purified in-house [19] was utilized for establishing a fluorescence-based screening assay using Gln-Ala-Arg peptide as the substrate. In this assay, the cleavage of AMC from Boc-Gln-Ala-Arg-7-amido-4 methylcoumarin hydrochloride (Boc-Gln-Ala-Arg-AMC) (Sigma, USA) was monitored by measuring the increase in fluorescence intensity of AMC released upon proteolytic cleavage at 480 nm (λ_{ex}= 360 nm). Boc-Gln-Ala-Arg-AMC was used as a substrate as described. The assay was standardized using published inhibitors [20,21].

Selectivity/safety screening assays with uPA, hepsin, factor Xa, thrombin, plasmin and trypsin were performed as described [19].

Cell Lines and Culture

Renca murine renal adenocarcinoma cell line was obtained from ATCC (American Type Culture Collection, catalog number CRL-2947), Manassas, VA and was cultured in RPMI-1640 with 10% fetal bovine serum. HUVEC cells used for the angiogenesis assay were cultured in endothelial Cell Growth Medium (Clonetics Catalog no. CC3024) with 2% Fetal Bovine Serum (JRH Catalog no. 12103-78P) and were used within three passages after the procurement. No further authentication was done for both Renca and HUVEC after the procurement. Both cell lines were cultured in a humidified atmosphere of 95% air and 5% CO_2 in an incubator at 37°C.

Antibodies and Western Blot Analysis

Human Matriptase/ST14 monoclonal antibody (Clone 416802) was purchased from R&D systems (Cat.No. MAB3946) and used at 1µg/ml for Western blot. Antibody to β-actin was purchased from Santa Cruz, Biotechnology (Cat.No. sc-69879). Cells were dissociated using Cell dissociation buffer (Invitrogen, Cat.No. 13151-014) and lysed using RIPA lysis buffer containing protease inhibitors at 20 µg/ml (Sigma cat no. P8340). Samples were run on 10% SDS-PAGE under reducing conditions and transferred to Polyvinylidene Difluoride (PVDF) membrane followed by probing with antibodies indicated above. Protein bands were visualized using horseradish peroxidase-labeled anti-rabbit IgG HRP secondary antibody (CST cat no.7074) and the ECL detection system (GE cat. no. RPN2232). Matriptase expression was determined across various epithelial cell lines by Western blot in order to validate matriptase expression in cell lines used for cell-based assays. A Western blot for β-actin (Catalog no. sc-69879) was performed in parallel as a loading control.

Cytotoxicity Assay

Cytotoxicity of the compounds was tested in cell lines using Calcein AM reagent (Sigma). The cells were seeded into 96-well plates and allowed to adhere for one day followed by addition of increasing concentrations of the test compound. After four days of incubation with the compound, cell culture medium was removed from the cells and Calcein AM reagent at 1µM final concentration was added (eBiosceince). The cells were then allowed to incubate at 37ºC for half an hour followed by reading on the spectrophotometer. Percent viability was calculated based on fluorescence value obtained at 485/520nm with cut off at 495nm.

Soft Agar Colony Formation Assay

In order to measure the consequence of inhibition of matriptase activity, colony forming potential of matriptase expressing cells was measured as described [22]. The cells were seeded in 0.7% nutrient agar with the test compound on an underlay

of 1.4% nutrient agar in a six well plate. On the day following the cell seeding, liquid cell culture medium containing the test compound was added to the wells to prevent the agar from drying out. Cell culture medium was changed regularly till the completion of the experiment. The cells were allowed to form colonies for a period of about three weeks following which the colonies were stained with 0.005% solution of crystal violet in 25% methanol. The colonies were counted under a dissecting microscope.

Invasion assay

Matrigel (BD Bioscience) was applied onto the upper chamber of an invasion insert with 8 µM pore size (BD Bioscience) and allowed to gel overnight. The lower chamber of a 24-well tissue culture plate (Corning) was filled with cell culture medium containing 10% FBS and 20 µg/ml fibronectin as chemoattractant for Renca cells. The cells were seeded into the upper chamber in 1% FBS containing-medium containing increasing concentrations of the test compound. The plate was incubated for about 3 days after which the invasion inserts were removed from the 24-well plate and non-invaded cells scraped off the side facing upper chamber of the insert. The chambers were stained with 0.5% crystal violet in methanol for one hour and allowed to dry after which the membranes were cut and mounted on slides for counting cells under a light microscope.

Migration Assay

Renca cells were seeded increasing concentrations of the test compound into trans well chambers and allowed to migrate for 24 hours using 10% FBS and 20 µg/ml fibronectin as chemoattractant. This was followed by fixing and staining of the trans well insert with 0.5% crystal violet in 25% methanol in order to visualize the migrated cells.

Evaluation of Matriptase Inhibitors on *In vitro* Angiogenesis

To detect inhibition of angiogenesis, the ability of the test compound to prevent tube formation by the HUVEC cells was monitored. The cells suspended in cell culture medium were seeded on matrigel in the presence or absence of matriptase inhibitor at 1 and 10 µM concentration. Following an incubation period of 6 hours at 37˚C in 5% CO_2, cells were stained with 2 µM calcein AM (Invitrogen) and pictures were taken by fluorescence microscopy.

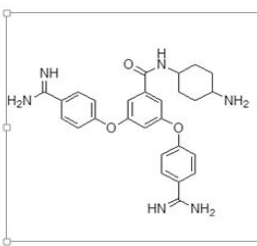
Orthotopic Mouse Renal Cell Carcinoma Model

Renca orthotopic xenograft model was established using 6-8 weeks-old Balb/c mice as described [23]. An incision on the skin and subcutaneous tissue at left lumbar region was made and the left kidney was exposed. Using a tuberculin syringe, 15,000 cells in 40 µl (20µl of medium + 20µl of Matrigel) per animal were injected into sub capsular area of the exposed kidney. The abdomen was closed with single stitch suture with silk and the skin was sutured in simple interrupted manner. After a recovery period of 2 days, dosing of Compound 15 was initiated at 0.05, 0.5 and 10 mg/kg doses once a day subcutaneously as single agent or at 0.5 mg/kg once a day subcutaneously in combination with 10 mg/kg of sorafenib dosed orally once a day. The vehicle used for Compound 15 was 2% ethanol and 10% hydroxyclcodextrin in phosphate buffered while vehicle for sorafenib was 12.5% Cremophore EL, 12.5% Ethanol, 75% autoclaved distilled water. Mortality of the animals, body weight loss and clinical symptoms were monitored daily. At the end of the dosing period, on the 23rd day, the animals were sacrificed, the tumor weights were calculated by subtracting the weight of the healthy right kidney from the cell-injected kidney. A portion of the tumor tissue was snap frozen for drug analysis and the rest was stored in 10% neutral buffered formalin for further histopathological processing. To detect metastasis, right inguinal lymph node, left inguinal lymph node, axillary, mediastinal, brachial, mesenteric lymph nodes, lung, liver, heart and bone (rib cage as well as femur and tibia) were stored in 10% neutral buffered formalin and histopathological sections were observed under microscope to quantify area covered by metastatic foci in the field analyzed by one-way ANOVA for the presence of metastatic foci. For determination of angiogenesis, CD31 immunostaining was performed on tumor sections to quantify blood vessel density.

Results

Compound-15 is a Selective Matriptase Inhibitor

Biochemical analysis demonstrated specific inhibition of matriptase by compound-15 (19) with a K_i value of 10 nM. The compound had a good selectivity profile against a panel of proteases as highlighted in (Figure 1) below.



Enzyme	K_i (µM) (fold selectivity)
Matriptase	0.01
uPA	>5.00 (>500x)
Hepsin	0.38 (38x)
Factor Xa	0.51 (51x)
Thrombin	2.10 (210x)
Plasmin	>5.00 (>500x)
Trypsin	1.62 (162x)

Figure 1: Structure and selectivity of compound-15, which was analyzed against indicated proteases in biochemical assays using appropriate substrates as described in "Materials and Methods".

Matriptase Expression in Cancer Cell Lines

Matriptase expression has been reported in several cell lines including Renca kidney cancer cell line and prostate cancer cell lines, PC3, LnCaP and DU145 [24]. Matriptase expression was evaluated in Renca kidney cancer cell line and compared to that

in prostate cancer cell lines as shown in (Figure 2A). Matriptase was found to be expressed in Renca cells to levels similar to that in prostate cancer cell lines.

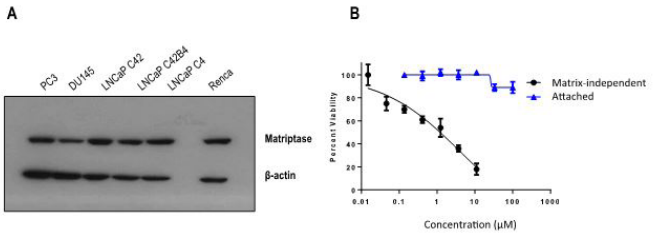


Figure 2: A. Relative expression of matriptase in Renca renal cell carcinoma and prostate cancer cell lines. Cell lysates from the indicated cells were subjected to immunoblot analysis using an antibody against human matriptase. **B**. Compound-15 exhibits anti-proliferative activity only when Renca cells are cultured in matrix-independent manner. Compound-15 was evaluated for its effect on proliferation of Renca cells grown as adherent monoloayer or in a matrix-independent manner on soft agar.

Compound-15 inhibits growth of Renca cells only when cultured in an anchorage-independent manner

Soft agar colony formation assay measures the long-term survival and anchorage-independent growth capacity of tumor cells. In order to determine the non-cytotoxic concentration of the test compound to be used in cell-based assays, the impact of compound-15 on cell viability of adherent Renca cells was studied. Compound-15 inhibited proliferation of adherent Renca cell line with an EC_{50} of 25.6 μM while it was more potent in soft agar colony formation assay where the EC_{50} of compound-15 was determined to be 2.8 μM. This demonstrated that matriptase inhibitor compound-15 has an impact on anti-proliferative activity only when Renca cells are cultured in matrix-independent manner as shown in (Figure 2B) above.

Impact of Matriptase Inhibitor on Migration, Invasion and Angiogenesis *in vitro*

Cell migration is a prerequisite for cancer invasion and metastasis, suggesting cell motility as a potential therapeutic target for cancer treatment. Matriptase is reported to enhance invasion through the HGF/cMet and uPA activation. Migration assays were performed using Renca cells to determine the effect of matriptase inhibition on cell motility. Compound-15 inhibited cell migration with an EC_{50} of 0.92 μM (Figure 3A).

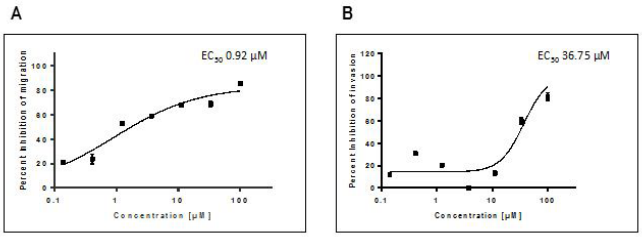


Figure 3: Inhibition of migration **(A)** and invasion (**B)** on treatment of Renca cells with compound-15, which was also evaluated for its effect on invasion and migration of Renca cells as described in "Materials and Methods".

Cell invasion assays were done to study the influence of compound-15 on cell migration through extracellular matrices, which occurs during cellular processes such as angiogenesis, embryonic development, immune response, and metastasis of cancer cells. This assay quantifies the degree to which invasive cells penetrate a barrier consisting of basement membrane components *in vitro* in response to chemo attractants and/or after treatment with test compounds. Compound-15 inhibited cell invasion of Renca cells with an EC_{50} of 36.75 μM (Figure 3B).

Compound-15 was also tested in an *in vitro* model for angiogenesis using Human Umbilical Vein Endothelial Cells (HUVEC) cells. Only treatment with a kinase inhibitor with potent inhibition of VEGF-R kinase (unpublished kinase inhibitor with potent inhibition of VEGF-R kinase) showed inhibition of tube formation by these primary vascular endothelial cells. Treatment with matriptase inhibitor compound-15 did not result in reduction in tube formation as shown in (Figure 4).

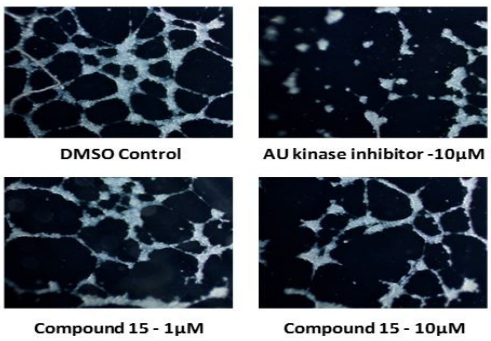


Figure 4: Lack of direct impact of matriptase inhibition on tube formation by HUVEC cells. As a measure of inhibition of angiogenesis, inhibition of tube formation by HUVEC cells was monitored upon growth of cells on Matrigel-coated plates as described in "Materials and Methods".

Efficacy of Compound-15 in Tumor Models

The in vivo efficacy of compound-15 was tested in Renca xenograft model. Renca cells readily establish tumors in isogenic mice to form adenocarcinoma that metastasizes within three weeks

[23].

In vivo pharmacokinetics data in mouse for compound-15 has been reported in an earlier publication. Compound-15 had a beta $t_{1/2}$ of 1.6 hours and AUC of 1000 ng*h/ml when dosed at 1 mg/kg by subcutaneous route as described earlier [25]. Prior to tumor growth inhibition studies, the maximum tolerated dose for compound-15 was determined to be 20 mg/kg when dosed subcutaneously once or twice a day dosing by subcutaneous route (data not shown). In the orthotopic Renca tumor model in Balb/C male mice, significant reduction in tumor weight was observed upon subcutaneous treatment with compound-15 at 0.5mg/kg ($p<0.01$) as a single agent or in combination with Sorafenib at 10mg/kg ($p<0.001$). Sorafenib alone also significantly reduced tumor volume ($p<0.001$). A dose dependent inhibition of tumor growth was observed with compound-15 administration (Figure 5A).

A dose dependent reduction in the number of lung tumor nodules was observed by treatment of animals with compound-15. An 80% reduction in lung tumor nodule formation was noted at 0.5 mg/kg dose of the compound-15 and the number of tumor nodules in lungs was further reduced with compound-15 at 0.5 mg/kg dosed in combination with Sorafenib at 10mg/kg ($p<0.001$). Sorafenib alone also reduced the number of lung nodules significantly when dosed at 10mg/kg ($p<0.01$) (Figure 5B). A histopathology study of lung tissue from this study confirmed reduction in metastatic foci upon treatment with these compounds and indicated potent inhibition of metastasis with compound-15 as shown in Figure 5C. The metastatic foci of similar size in both compound-15 and sorafenib treatment groups (Figure 5C) suggest inhibition of metastasis rather than proliferation due to treatment with matriptase inhibitor.

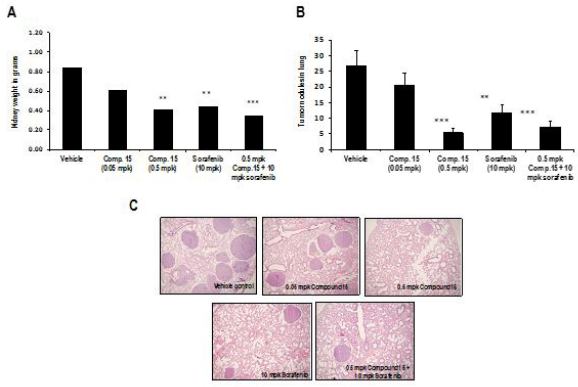


Figure 5: Compound-15 is efficacious in an orthotopic Renca tumor model. A. Impact of matriptase inhibition on primary tumor growth in renal cell carcinoma model. Error bars represent SEM. Analysis of variance with one factor (treatment) over time. Stars on the bars represent **p** value when compared to vehicle control. ** $p<0.01$, *** $p<0.001$. B. Impact of matriptase inhibition on tumor nodules in the lung. Error bars represent SEM. Analysis of variance with one factor (treatment) over time. Stars on the bars represent p value when compared to vehicle control. ** $p<0.01$, *** $p<0.001$. C. Histopathology of lung from representative tumors.

In addition to having an effect on tumor growth and metastasis, compound-15 was also found to inhibit angiogenesis as shown in (Figure 6). Cluster of differentiation 31 (CD31), also known as Platelet Endothelial Cell Adhesion Molecule (PECAM-1), a molecule involved in angiogenesis, [26] was stained to quantify vessel density. Compound-15 showed excellent inhibition of angiogenesis at 0.5 mg/kg and the observed effect was comparable to that obtained with 10 mg/kg Sorafenib. Importantly, number of vessels per field, as measured by CD31 staining, was significantly lower in the group of animals treated with a combination of compound-15 at 0.5 mg/Kg and Sorafenib at 10 mg/Kg when compared to 10 mg/Kg sorafenib administered as a single agent.

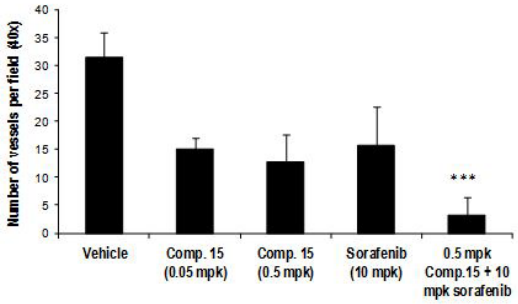


Figure 6: Impact of matriptase inhibition on blood vessel density in Renca tumors. Quantification of blood vessel density in Renca tumors as observed by CD31 immunostaining. Error bars represent SEM. Analysis of variance with one factor (treatment) over time. Asterisks on the bars represent p value when compared to vehicle control. Comp: compound; *** $p<0.001$

Discussion

In this study, we report the efficacy of a recently described specific matriptase inhibitor, compound-15, in an orthotopic Renca xenograft tumor model. The compound demonstrated a strong dose-dependent inhibition of tumor growth. Tumor growth reduction is consistent with the significant inhibition of proliferation *in vitro* when Renca cells were cultured in a matrix-independent manner but not when grown as a regular monolayer culture. Apart from demonstrating tumor growth inhibition, compound-15 was found to inhibit lung metastasis in Renca model both as single agent as well as in combination with the approved anti-angiogenic drug sorafenib. In combination with sorafenib, tumor growth inhibition was greater than when compared to either agent alone. Since the metastatic foci in the lung were found to be of similar size in both the high and low dose treatment groups of compound-15, inhibition of metastasis rather than proliferation is possibly the primary mechanism of action of the matriptase inhibitor in this model. This was in agreement with our *in vitro* data that confirmed

the impact of this compound on migration and invasion of the Renca cell line. Immunostaining for CD31 was done to quantify vessel density in tumors. Inhibition of angiogenesis was observed at 0.5 mg/kg of compound-15 and the effect was comparable to that obtained with 10 mg/kg sorafenib. Inhibition of angiogenesis by compound-15 may be responsible for it's impact on metastasis. Furthermore, an additive effect was seen with a combination of 0.5 mg/kg of compound-15 with sorafenib. These results suggest that, in renal cancer, treatment with a combination of a matriptase inhibitor and sorafenib could be more efficacious than treatment with sorafenib alone.

Our results are the first direct demonstration of the antiangiogenic potential of matriptase in a tumor model of renal origin. We propose that the following sequence of events, impacted by matriptase inhibition, could occur during tumor development. Matriptase inhibitor reduces the primary tumor growth because of the effect of the inhibitor on matrix-independent growth as demonstrated in this study. Additionally, the matriptase inhibition results in a reduction in migration and invasive capacity of the tumor cells. A recent study has described matriptase expression in RCC bone metastases, where it is more highly expressed than in the RCC primary sites. This was shown to be accompanied by matriptase expression in osteoclasts indicating its importance in bone metastasis [27]. Small matriptase expressing, avascular tumors promote endothelial cell migration, differentiation and morphogenesis in microvascular endothelial cells upon shedding of matriptase due to hypoxia [14]. During tumor progression matriptase upregulation induced by TGF-β leads to tumor growth and EMT transition which results in formation of large, highly vascularized tumors which can metastasize using these blood vessels [28]. The inhibition of matriptase by compound-15 may prevent neovascularization of the tumor and induce vascular regression resulting in inhibition of tumor growth and metastasis.

Inhibition of angiogenesis in the orthotropic Renca models is consistent with the previous reports in which matriptase transfected human gastric cancer cell line, AZ521 cells grew faster and produced much larger tumors with significantly higher number and larger size of blood vessels in tumor tissue, proposed to be partially mediated through activation of matrix metalloproteinases, stromelysin (MMP-3) [15].

A role of matriptase inhibition in reduction of tumor growth and metastasis formation is in agreement with the reports in which CVS-3983, a selective matriptase inhibitor, has been found to suppress the growth of androgen independent CWR22R and CWRSA6 prostate tumor xenografts [16]. In another study, bis-basic secondary amides of sulfonylated 3-amidinophenylalanine matriptase inhibitors were found to reduce both the primary tumor growth, as well as tumor dissemination in an orthotopic PC3 prostate cancer xenograft mouse model [29].

The Renca orthotopic renal cancer model is most commonly used for assessment of the efficacy of various therapies for renal cell carcinoma. Renca cells readily establish tumors in isogenic mice to form adenocarcinoma that similar to clinical situation metastasizes to distant organs [23]. The significant anti-tumor activity in the Renca model observed with a selective matriptase inhibitor supports further development of matriptase inhibitors for renal cancer therapy. Additionally, the enhanced anti-tumor activity observed when a matriptase inhibitor is combined with sorafenib, an agent approved for use in renal cell carcinoma, suggests that a combination of matriptase inhibitor with sorafenib in renal cell carcinoma therapy could be more efficacious than treatment with either agent alone.

In this study we demonstrated that compound-15 can inhibit renal cell proliferation in a matrix-independent manner as well as cell migration and invasion. In the orthotopic Renca kidney cancer model, compound-15 treatment resulted in inhibition of primary tumor growth and metastasis, which correlated well with inhibition of angiogenesis. An additive effect on tumor growth and metastasis was observed when compound-15 was combined with sorafenib, a kinase inhibitor. Preclinical evidence presented here provides a rationale for inhibition of matriptase with or without sorafenib as a therapeutic modality in renal cancer.

Acknowledgments: We gratefully acknowledge Orion Corporation for funding this research.

Disclosure of Potential Conflicts of Interest: The authors are employees of Aurigene Discovery Technologies Limited or Orion Corporation as shown.

References

1. Oberst MD, Johnson MD, Dickson RB, Lin CY, Singh B, et al. (2002) Expression of the serine protease matriptase and its inhibitor HAI-1 in epithelial ovarian cancer: correlation with clinical outcome and tumor clinicopathological parameters. Clin Cancer Res 8: 1101-1107.

2. Szabo R, Hobson JP, Christoph K, Kosa P, List K, et al. (2009) Regulation of cell surface protease matriptase by HAI2 is essential for placental development, neural tube closure and embryonic survival in mice. Development 136: 2653-2663.

3. Tanimoto H, Underwood LJ, Wang Y, Shigemasa K, Parmley TH, et al. (2001) Ovarian tumor cells express a transmembrane serine protease: a potential candidate for early diagnosis and therapeutic intervention. Tumour Biol 22: 104-114.

4. Benaud CM, Oberst M, Dickson RB, Lin CY (2002) Deregulated activation of matriptase in breast cancer cells. Clin Exp Metastasis 19: 639-649.

5. Saleem M, Adhami VM, Zhong W, Longley BJ, Lin CY, et al. (2006) A novel biomarker for staging human prostate adenocarcinoma: overexpression of matriptase with concomitant loss of its inhibitor, hepatocyte growth factor activator inhibitor-1. Cancer Epidemiol Biomarkers Prev 15: 217-227.

6. Wu SR, Cheng TS, Chen WC, Shyu HY, Ko CJ, et al. (2010) Matriptase is involved in ErbB-2-induced prostate cancer cell invasion. Am J Pathol 177: 3145-3158.

7. List K, Haudenschild CC, Szabo R, Chen WJ, Wahl SM, et al. (2002) Matriptase/MT-SP1 is required for postnatal survival, epidermal barrier function, hair follicle development, and thymic homeostasis. Oncogene 21: 3765-3779.

8. Lee SL, Dickson RB, Lin CY (2000) Activation of hepatocyte growth factor and urokinase/plasminogen activator by matriptase, an epithelial membrane serine protease. J Biol Chem 275: 36720-36725.

9. Takeuchi T, Harris JL, Huang W, Yan KW, Coughlin SR, et al. (2000) Cellular localization of membrane-type serine protease 1 and identification of protease activated receptor-2 and single-chain urokinase-type plasminogen activator as substrates. J Biol Chem 275: 26333-26342.

10. Ustach CV, Huang W, Conley-LaComb MK, Lin C-Y, Mingxin C, et al. (2010) A novel signaling axis of matriptase/PDGF-D/β-PDGFR in human prostate cancer. Cancer Res 70: 9631-9640.

11. Mika S, Hiroshi K, Naohiro K, Yasushi S, Mitsuaki S, et al. (2004) Inhibition of Tumor Invasion by genomic down-regulation of matriptase through suppression of activation of receptor-bound pro-urokinase. J Biol Chem 279:14899 -14908.

12. List K, Szabo R, Molinolo A, Sriuranpong V, Redeye V, et al. (2005) Deregulated matriptase causes ras-independent multistage carcinogenesis and promotes ras-mediated malignant transformation. Genes Dev 19: 1934-1950.

13. Uhland K (2006) Matriptase and its putative role in cancer. Cell Mol Life Sci 63: 2968-2978.

14. Kim SB, Lee D, Jeong JW, Kim C, Park D, et al. (2010) Soluble Epithin/PRSS14 Secreted from Cancer Cells Contains Active Angiogenic Potential. Mol Cells 29: 617-623.

15. Jin X, Yagi M, Akiyama N, Hirosaki T, Higashi S, et al. (2006) Matriptase activates stromelysin (MMP-3) and promotes tumor growth and angiogenesis. Cancer Sci 97: 1327-1334.

16. Galkin AV, Mullen L, Fox WD, Brown J, Duncan D, et al. (2004) CVS-3983, a Selective matriptase inhibitor, suppresses the growth of androgen independent prostate tumor xenografts. Prostate 61: 228-235.

17. Verheul HMW, Hammers H, Erp KV, Wei Y, Sanni T, et al. (2007) Vascular endothelial growth factor trap blocks tumor growth, metastasis formation, and vascular leakage in an orthotopic murine renal cell cancer model. Clin Cancer Res 13: 4201-4208.

18. Jin JS, Chen A, Hsieh DS, Yao CW, Cheng MF, et al. (2006) Expression of serine protease matriptase in renal cell carcinoma: correlation of tissue microarray immunohistochemical expression analysis results with clinicopathological parameters. Int J Surg Pathol 14: 65-72.

19. Goswami R, Mukherjee S, Wohlfarht G, Ghadiyaram G, Nagraj J, et al. (2013) Discovery of pyridyl Bis(oxy)dibenzimidamide derivatives as selective matriptase Inhibitors. ACS Med Chem Lett 4: 1152-1157.

20. Steinmetzer T, Schweinitz A, Stürzebecher A, Dönnecke D, Uhland K, et al. (2006) Secondary amides of sulfonylated 3-amidinophenylalanine. New potent and selective inhibitors of matriptase. J Med Chem 49: 4116-4126.

21. Enyedy IJ, Lee SL, Kuo AH, Dickson RB, Lin CY, et al. (2001) Structure-based approach for the discovery of bis-benzamidines as novel inhibitors of matriptase. J Med Chem 26: 44: 1349-1355.

22. Shappell SB, Gupta RA, Manning S, Whitehead R, Boeglin WE, et al. (2001) 15S-Hydroxyeicosatetraenoic acid activates peroxisome proliferator-activated receptor gamma and inhibits proliferation in PC3 prostate carcinoma cells. Cancer Res 61: 497-503.

23. Nishisaka N, Morse P, Jones RF, Wang CY, Haas GP (2001) Murine animal model. Methods Mol Med. 53: 255-264.

24. Saleem M, Adhami VM, Zhong W, Longley BJ, Lin CY, et al. (2006) A novel biomarker for staging human prostate adenocarcinoma: overexpression of matriptase with concomitant loss of its inhibitor, hepatocyte growth factor activator inhibitor-1. Cancer Epidemiol Biomarkers Prev 15: 217-227.

25. Goswami R, Mukherjee S, Ghadiyaram C, Wohlfahrt G, Sistla RK, et al. (2014) Structure-Guided Discovery of 1,3,5 Tri-Substituted Benzenes as Potent & Selective Matriptase Inhibitors Exhibiting in Vivo Antitumor Efficacy. Bioorg Med Chem 22: 3187-3203.

26. DeLisser HM, Christofidou-Solomidou M, Strieter RM, Burdick MD, Robinson CS, et al. (1997) Involvement of endothelial PECAM-1/CD31 in angiogenesis. Am J Path 151: 671-677.

27. Mukai S, Yorita K, Kawagoe Y, Katayama Y, Nakahara K, et al. (2015) Matriptase and MET are prominently expressed at the site of bone metastasis in renal cell carcinoma: immunohistochemical analysis. Human Cell 28: 44-50.

28. Lee HS, Kim C, Kim SB, Kim MG, Park D (2010) Epithin, a target of transforming growth factor-β mediates epithelial to mesenchymal transition. Biochem Biophys Res Commun 395: 553-559.

29. Steinmetzer T, Schweinitz A, Stu¨rzebecher A, Do¨nnecke D, Uhland K, et al. (2006) Secondary amides of sulfonylated 3-amidinophenylalanine. New potent and selective inhibitors of matriptase. J Med Chem 49: 4116-4126.