Antibody-Mediated Inhibition of Cathepsin S Blocks Colorectal Tumor Invasion and Angiogenesis

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Abstract

Purpose: Cathepsin S is a cysteine protease that promotes the invasion of tumor and endothelial cells during cancer progression. Here we investigated the potential to target cathepsin S using an antagonistic antibody, Fsn0503, to block these tumorigenic effects.

Experimental Design: A panel of monoclonal antibodies was raised to human cathepsin S. The effects of a selected antibody were subsequently determined using invasion and proteolysis assays. Endothelial cell tube formation and aorta sprouting assays were done to examine antiangiogenic effects. In vivo effects were also evaluated using HCT116 xenograft studies.

Results: A selected cathepsin S antibody, Fsn0503, significantly blocked invasion of a range of tumor cell lines, most significantly HCT116 colorectal carcinoma cells, through inhibition of extracellular cathepsin S–mediated proteolysis. We subsequently found enhanced expression of cathepsin S in colorectal adenocarcinoma biopsies when compared with normal colon tissue. Moreover, Fsn0503 blocked endothelial cell capillary tube formation and aortic microvascular sprouting. We further showed that administration of Fsn0503 resulted in inhibition of tumor growth and neovascularization of HCT116 xenograft tumors.

Conclusions: These results show that blocking the invasive and proangiogenic effects of cathepsin S with antibody inhibitors may have therapeutic utility upon further preclinical and clinical evaluation. (Clin Cancer Res 2009;15(19):6042–51)

The lysosomal cysteine cathepsins encompass a family of closely related cysteine proteases, mediating a diverse range of proteolytic effects (1–4). However, an increasing body of evidence has shown the overexpression of a number of cysteine cathepsins in cancer (5–7). Significantly, these proteases are secreted into the tumor extracellular milieu, producing potent degradative effects on a broad range of extracellular matrix (ECM) components, including collagen and laminins (8–10). Further confirmation of these effects were provided in a murine model of sporadic pancreatic carcinogenesis (RIP1-Tag2), in which the genetic ablation of either cathepsin B or cathepsin S severally or cathepsin B deficiency inhibited tumor proliferation (11). These observations highlight their potential as therapeutic targets in cancer treatment. Indeed, the application of synthetic broad-spectrum probes and combination therapies has successfully shown efficacy in vivo using various tumor models (12–15). However, given the roles that these proteases play in normal cellular homeostasis, an approach that selectively targets a specific cathepsin with limited normal tissue distribution may be more therapeutically attractive

Cathepsin S, unlike the ubiquitous cathepsin B and cathepsin L, exhibits a restricted tissue expression. It is found predominantly in lymphatic tissue, macrophages, and other professional antigen-presenting cells (16); mediating key steps in antigen presentation through cleavage of the invariant chain (17, 18). However, the inappropriate expression of cathepsin S has also been observed in a range of tumors such as astrocytomas (19–21), prostate (22), hepatocellular (23), and pancreatic carcinomas (11). Crucially, evidence from the RIP1-Tag2 model and other studies (11, 23–25) also suggests a pivotal role for endothelium-derived cathepsin S in neovascularization.

In this current investigation we describe the development of an antibody to specifically bind and attenuate cathepsin S over other closely related cathepsins. We show that application of this antibody, Fsn0503, blocks tumor cell invasion through ablation of cathepsin S–mediated ECM remodeling. Furthermore, Fsn0503 also inhibits endothelial tube formation and
translational relevance

This investigation shows the ability to target and block cathepsin S, expressed at elevated levels in the microenvironment of colorectal carcinomas, using an antagonistic antibody. The application of an antibody inhibitor to cathepsin S, Fsn0503, represents a new modality for cancer treatment that not only blocks the invasion of tumors, but also targets neovascularization. Furthermore, its selectivity to cathepsin S and preferential targeting to extracellular pathologic cathepsin S may circumvent off-target effects. We believe that this antibody could have clinical utility in the treatment of colorectal and other invasive tumors in which elevated levels of cathepsin S have been identified. The combination of chemotherapy treatments with an anti-invasive/antiangiogenic agent such as Fsn0503 could lead to improved clinical outcomes in aggressive and metastatic tumors.

development of cathepsin S antibodies

Cathepsin S monoclonal antibodies were generated by immunization of BALB/c mice with recombinant mature human cathepsin S residues 115-331 using standard protocols. Splenic B-cells from antigen-reactive mice were fused with SP2 myeloma cells, and the resultant hybridoma clones were analyzed for secretion of cathepsin S antibodies by ELISA. Antibodies from positive hybridomas were affinity-purified using protein A, desalted into PBS, and final concentration was determined by the bicinchoninic acid assay (Pierce).

Live-cell proteolysis assay

Precooled glass coverslips were coated with 25 μg/mL of quenched fluorescent substrate, DQ-Gelatin (Molecular Probes), in a 2% gelatin/2% sucrose solution in PBS and incubated on ice for 15 min to solidify. U251MG and HCT116 cells (20,000) were seeded onto each coverslip and incubated at 37°C for 1 h (in DMEM or McCoy’s 5A media, respectively). The cells were then incubated in appropriate supplemented media with either cathepsin S or isotype control antibodies (250-300 nmol/L). Cells were incubated for 24 h at 37°C with 5% CO₂ and fluorescent degradation products observed using a Leica SP2 AOBs confocal microscope, with ×40 oil immersion objective.

ELISA

Recombinant human cathepsins S, L, K, and B were coated onto a 96-well plate (100 ng) and incubated with varying concentrations of Fsn0503. After rinsing with PBS-Tween and incubation with goat anti-mouse horseradish peroxidase–conjugated secondary antibody, wells were incubated with the chromogenic substrate tetramethylbenzidine (Calbiochem) for 10 min at room temperature. The reaction was stopped by the addition of 500 mmol/L HCl, and absorbance was read at 450 nm.

Affinity binding measurements

Quantitative analysis and determination of the kinetic dissociation constant was determined using a Biacore 3000 surface plasmon resonance instrument. Measurement of binding kinetics was achieved by injecting 20 nmol/L of Fsn0503 for 2 min at 30 μL/min, yielding immobilization levels of 324 to 330 relative units. After capturing of antibody, varying concentrations of cathepsin S antigen ranging from 7.8 mmol/L to 1 μmol/L were analyzed (5-min association time, 10-min dissociation time, at a flow rate of 30 μL/min) in 10 mmol/mL HEPES pH 7.4, containing 150 mmol/L NaCl and 0.005% Tween 20. Kinetic constants were calculated using BIAevaluation software version 4.1. Separate fits of the initial phase of dissociation and association were done using a 1:1 Langmuir binding model.

in vitro invasion assays

In vitro invasion assays were done as previously described (26). Cells were seeded in serum-free media in the presence of anti-cathepsin S or control antibodies.

Immunocytochemistry

Cells were grown on glass coverslips, fixed using ice-cold acetone, blocked in 10% normal goat serum, and incubated with the cathepsin S antibody (10 μg/mL). Coverslips were rinsed with PBS and incubated with antimouse AlexaFluor 488–labeled secondary antibody (4 μg/mL) and counterstained by incubation with Rhodamine phalloidin (Invitrogen) for 15 min. PBS-rinsed coverslips were mounted using PermaFluor mounting media and visualized by fluorescence microscopy.

Immunohistochemistry

Serial sections of the AccuMax Colo066 tissue microarray were stained for cathepsin S and CD68. Arrays were dewaxed according to the manufacturer’s instructions and stained on the Ventana Discovery platform using the UltraMap detection system under local ethics permission (ORECNI 08/NIR03/122) using cathepsin S (7 μg/mL) and anti-CD68 (Dako, 0.5 μg/mL) antibodies. Bound primary antibody was visualized using goat anti-mouse horseradish peroxidase (Abcam, 5 μg/mL). Freshly cut xenograft tumor sections were stained for CD34 using the Vectastain ABC detection system. Microwave antigen retrieval in citrate buffer was done. Antimouse CD34 (Abcam; MEC14.7 clone, 2 μg/mL) and biotinylated antirat secondary antibody (Vectolabs, 10 μg/mL). All slides were counterstained with hematoxylin.

Endothelial tube formation assay

Human umbilical vein endothelial cells were counted and resuspended in MCDB-131 growth medium, on Matrigel-coated 48-well plates (10 mg/mL) containing Fsn0503 or isotype control. Cells were incubated with vehicle-only control medium containing the appropriate volumes of PBS. After incubation at 37°C and 5% CO₂ for 24 h, cells were viewed using a Nikon Eclipse TE300 microscope (×20). Total tubule branching was counted and results were expressed as a percentage relative to the control. All studies were done in triplicate and 10 images were taken from each replicate before analysis.

Rat aorta arch assay

The aorta from an 8-week-old Wistar rat was dissected and sectioned into 1-mm rings and washed in culture medium containing antibiotics. Media were aspirated from the 24-well plate and a single ring was placed onto rehydrated Matrigel thin-layer 24-well plates (BD Biosciences). The rings were overlaid with 300 μL of Matrigel (diluted 1:3 in media). The plate was incubated at 37°C with 5% CO₂ for 30 min, after which the antibodies were applied in 1 mL of media (final concentration range of 1 × 10⁻⁴ to 1 × 10⁻⁶ μg/mL). Plates were incubated in 5% CO₂ at 37°C for 7 d to allow formation of tubules from the aortic ring. After 7 d, tube formation was quantified using a Nikon Eclipse TE300 microscope, with an integrated Nikon DXM1200 digital camera.

In vivo xenograft evaluation

Six- to eight-week-old female BALB/c nude mice were implanted with 2 × 10⁸ HCT116 cells s.c. on either flank. Cells were prepared by harvesting in the log phase of growth; they were washed in HBSS and mixed 1:1 with BD Basement Membrane Matrigel mix. The mice were randomly separated into treatment groups (n = 10) on the day of implantation. The mice were treated with 10 mg/kg anti-cathepsin S or isotype control 3 times per week (s.c., i.p., or i.v.). Tumor measurements were taken using digital calipers. All tumors and mouse organs were excised at study termination and formalin-fixed for histopathology. All animal experiments were carried out in accordance with the Animal (Scientific Procedures) Act 1986 and conformed to current UK Co-ordinating Committee on Cancer Research guidelines.
Statistical analysis. Statistical analyses were done on experimental results using Student’s t test of variance. All experiments were repeated a minimum of three times and data are expressed as means ± SD.

Results

Isolation and characterization of antagonistic cathepsin S antibody. The first aim of this study was the isolation and application of antibodies towards cathepsin S that would circumvent specificity problems associated with small molecule approaches (5, 26–28). We developed a panel of murine monoclonal antibodies using recombinant mature human cathepsin S protein as immunogen. Following limiting dilutions, individual clones were screened for their ability to bind the cathepsin S antigen over a control antigen (recombinant human leucocyte-specific tyrosine kinase) purified under identical conditions. As can be seen in Fig. 1A, antibodies from individual clone supernatants showed clear specificity towards the cathepsin S antigen as anticipated.

Previously, we showed that the inhibition of cathepsin S–like activity, using a peptidyl inhibitor leucine homophenylalanine vinyl sulfone (LHVS), attenuated astrocytoma tumor cell invasion (19). Therefore, we screened purified monoclonal antibodies for the ability to similarly attenuate invasion of U251MG cells, observing inhibition of invasion with several individual clones, the most potent of which reduced invasion by 50% at 200 nmol/L (Fig. 1B), suggesting that the antibody limited the progression of the cells through the ECM.

Cathepsin S has been shown to efficiently degrade ECM components such as collagen and gelatin in vitro (29, 30), and we wished to determine if this selected cathepsin S antibody (subsequently referred to as Fsn0503) could block this hydrolysis in cell-based assays. Live-cell proteolysis assays were prepared by incubation of U251MG cells in the presence of DQ-gelatin, representative of denatured collagen. This resulted in circumferential cellular fluorescence, caused by the degradation and release of the fluorophore by secreted proteases (Fig. 1C, top; ref. 31). However, in the presence of Fsn0503 (300 nmol/L), this degradation was marked and consistently impaired over an isotype control antibody (Fig. 1C, middle and bottom), indicating that the antibody had limited the proteolysis of the DQ-gelatin.

To confirm that the effects of Fsn0503 were due to its binding to cathepsin S and not other closely related cathepsins, its selectivity was determined by ELISA, in which it exhibited an expected sigmoidal binding profile at concentrations as low as 1 × 10⁻¹⁰ mol/L to immobilized cognate antigen (recombinant cathepsin S; Fig. 2A). Crucially, no detectable interaction with recombinant human cathepsins K, L, or B was observed. We further analyzed antibody binding to the panel of cathepsins by Western blot, and observed that the antibody bound specifically to cathepsin S (Fig. 2B). Finally, the isotype of Fsn0503 was determined as an IgG1 subclass (data not shown).

Tumors can exhibit marginally acidic pH in their microenvironments (32), and it has recently been shown that this acidification can promote the invasive effects attributable to cathepsin S in the tumor microenvironment (33). To ascertain that Fsn0503 could maintain its binding to cathepsin S at potential acidic extracellular pH levels, binding analysis by ELISA was done. It was found that Fsn0503 bound the immobilized cathepsin S with comparable affinities over a pH range of 5.5
to 7.5, and exhibited only slightly reduced binding at the lower pH 4.9 (Fig. 2C).

Finally, the binding affinity of the antibody was quantified by plasmon surface resonance. Separate fits of the initial phases of dissociation and association were done using a 1:1 Langmuir binding model providing elucidation of the KD at 2.8 (± 0.3) nmol/L \([k_{\text{ass}} = 4.4 (± 0.8) \times 10^3 \text{ M}^{-1}\text{s}^{-1} \text{ and } k_{\text{diss}} = 1.2 (± 0.09) \times 10^{-5} \text{ s}^{-1}\]) (Fig. 2D). Collectively, these data show the selectivity and potency of Fsn0503 to cathepsin S.

Fsn0503 blocks colorectal carcinoma cell invasion. The application of Fsn0503 had been validated using the astrocytoma cell line U251MG. Next we determined the effect of Fsn0503 towards other cell lines, representative of the major classes of aggressive tumors, in which we previously showed expression of cathepsin S (34). The most significant effects were obtained against the colorectal carcinoma cell line HCT116 (at 200 nmol/L) in which a 64% reduction \((P \leq 0.0001)\) was observed (Fig. 3A). Higher concentrations of antibody produced no further significant reductions in invasion (data not shown). This was not unexpected due to the presence of other proteases in the tumor microenvironment. Fsn0503 also blocked the ability of prostate PC3 and breast MDA-MB-231 cell lines to invade in the presence of identical concentrations (34% and 32%, respectively; \(P \leq 0.0001\); Fig. 3B). To confirm that the action of the antibody was an anti-invasive effect rather than a cytotoxic mechanism, MTT cell viability assays were carried out. No appreciable alteration in cell viability was observed, confirming that the antibody directly attenuated the invasive potential of the cells (data not shown).

Immunocytochemical analysis of the HCT116 cells allowed further analysis of the expression of cathepsin S in these cells (Fig. 3C). As anticipated, punctuate localization, consistent with lysosomal localization, was observed. Additionally, colocalization of actin and cathepsin S (as indicated by arrows) suggested a proportion of this protease was also present at the cell surface, consistent with secretion and cell surface localization. We then examined the ability of these cells to hydrolyze DQ-gelatin and observed circumferential cellular fluorescence, which was significantly reduced by 86% in the presence of Fsn0503 (250 nmol/L) over an isotype control \((P < 0.0006)\). This suggested that the cleavage of collagen-derived components of the ECM was highly dependent on cathepsin S in the HCT116 cells. Furthermore, this may explain the extent of invasion attenuation observed for this cell line, given that the experiments were done using artificial ECM (Matrigel) of which collagen is a major component.

Cathepsin S is overexpressed in colorectal carcinomas. On the basis of the findings with these cell lines, we examined further the expression of cathepsin S in colorectal cancers as we had already observed elevated expression of this protease in astrocytomas (19–21) and upregulation had already been observed in prostate cancer (22). Cathepsin S expression was determined in the Accu-max Colo066 tissue microarray, containing a range of nonneoplastic colorectal mucosa, \((n = 8)\) and colorectal cancer samples \((n = 45)\). As shown in Fig. 4A, cytoplasmic cathepsin S staining was seen in normal colorectal epithelium. However, cytoplasmic staining was intensified in the different grades of colorectal tumor, indicating increased expression of the protease.
Moreover, granularity was observed in the neoplastic epithelium, consistent with anticipated lysosomal localization of the protease. Previously it was observed that infiltrating macrophages are a source of cathepsin S in the tumor microenvironment (19). Furthermore, others have shown that this source of the enzyme can contribute significantly to the progression of the disease (14). Therefore, we examined parallel sections of normal colorectal mucosa and colorectal tumor samples for the presence of macrophages using CD68 staining (Fig. 4B). Although epithelial-associated and macrophage-associated cathepsin S expression was identified in the nonneoplastic tissue, this pattern was less obvious in tumors, due to an increased intensity of staining for cathepsin S in the tumor tissue.

**Fsn0503 blocks angiogenesis.** A phenotypic characteristic of murine cathepsin S knockout animals is impaired microvessel formation and neovascularization (24, 25). Endothelial tube formation is an early process in angiogenesis whereby endothelial cells form distinct joining structures between colonies on a supporting matrix (35). As shown in Fig. 4B, the tube networks formed normally. However, in the presence of Fsn0503 (200 nmol/L), this process was significantly disrupted with a 63% reduction in tube formation (Fig. 5B; P < 0.01). In addition, the antibody

![Fig. 3.](image-url)
inhibited human umbilical vein endothelial cell invasion using the Matrigel Boyden chamber assay as described in Fig. 1 (data not shown). Thus, in agreement with previous findings, we conclude that cathepsin S promotes the localized invasion and movement of the endothelial cells during tube formation, and that this process is blocked by Fsn0503.

Next, the application of Fsn0503 towards tube sprouting in the rat aorta arch assay was examined. The vessel outgrowth from aortic rings was analyzed and quantified in terms of tube number, mean and maximum tube length. Incubation of the aorta rings in the presence of Fsn0503 resulted in a dose-dependent reduction in tubule formation (up to 70%) in comparison with an isotype control (Fig. 5C and D). Collectively the results from these models clearly show the ability of Fsn0503 to attenuate angiogenesis through abrogation of tube formation and microvascular sprouting.

**Fsn0503 blocks tumor neovascularization and tumor development in vivo.** Given that Fsn0503 inhibited the movement of both tumor and endothelial cells, it was investigated if these effects could also be observed in vivo. HCT116 xenografts in Balb/c nude mice were established s.c. and treated with the cathepsin S antibody (10 mg/kg, 3 times per week). As shown in Fig. 6A, administration of the antibody s.c. resulted in significant reduction of tumor development, approaching 50% after 26 days over an isotype control (P < 0.001). Similar tumor reductions were observed whether the antibody was administered i.p., i.v., or s.c. (data not shown). Subsequent immunohistochemical analysis showed that the endothelial marker CD34 (as highlighted with arrows on the sections) was markedly reduced in the Fsn0503-treated group (Fig. 6B). This was indicative of impaired endothelial cell infiltration and associated tumor neovascularization, consistent with the impaired tumor development observed.

**Discussion**

In this current work, the potential of cathepsin S as a tumor target amendable to antibody treatment has been examined. The results show that a monoclonal antibody, Fsn0503, selectively targeted cathepsin S, inhibiting the breakdown of ECM degradation around the extracellular periphery of tumor cells. This resulted in the attenuation of tumor cell invasion through the ECM as shown using in vitro invasion assays. These anti-invasive properties were observed across a range of tumor cell lines, but the most significant effects were found towards the colorectal carcinoma cell line HCT116. This led to a further examination of a panel of colorectal carcinoma biopsies and revealed upregulation of the protease in the diseased tissue, suggestive of an important role in colorectal tumor development.

There is an increasing body of data highlighting the upregulation of cathepsin S in a spectrum of tumors (11, 22, 23), particularly in astrocytomas and glioblastomas, where levels of the protease increase with the grade and aggressiveness (and associated poorer outcome) of disease (20, 21). These investigations examined the source of the protease and showed that it came predominantly from the tumor cells, with only a proportion attributable to infiltrating immune cells, an abundant source of cathepsin S. Similarly, here we observed macrophages expressing cathepsin S in colorectal tumors, but it was clear that the neoplastic cells were the predominant source of the protease. These findings

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**Fig. 4.** Cathepsin S is upregulated in colorectal adenocarcinomas. A, increasing intensity of cathepsin S staining in colorectal samples; i, cytoplasmic staining in nonneoplastic tissue; ii-v, increasing intensity of staining in neoplastic tissue. B, moderately differentiated tumor with cytoplasmic staining of cathepsin S (left) when parallel-stained for CD68 (right) shows a small number of macrophages (arrows), suggesting that the majority of cathepsin S staining is derived from neoplastic epithelium.
are in contrast to studies in murine tumor models (RIP1-Tag2) where it has been shown that in the pancreatic islet tumors, the infiltrating immune cells seem to be the major source of cathepsin S (14). These differences could be attributable to the different tumor types examined or species variation in cathepsin function.

In addition to confirming a causative role for cathepsin S in tumor cell invasion, murine studies have also shown that endothelial-derived cathepsin S promotes angiogenesis. In cathepsin S null mice, it has been shown that endothelial cells are less invasive, resulting in attenuation of their ability to migrate and form tubes during early angiogenesis (18). Similarly, the genetic ablation of cathepsin S in the RIP1-Tag2 model resulted in reduced angiogenic pancreatic islets and reduced tumor neovascularization (11). Further analysis of the RIP1-Tag2 animals also suggests that cathepsin S promotes these effects through modulation of angiogenic signaling factors (25). Additionally, gene expression analysis of a hepatocellular carcinoma murine model showed that cathepsin S was the most significantly upregulated protease in endothelial cells during vessel formation (23). Here, in support of these observations, it has been shown that Fsn0503 blocks angiogenesis at least in part by inhibition of tube formation and endothelial infiltration into developing tumors.

Cathepsin S is only one of a number of proteases that have been implicated in the remodeling of the tumor microenvironment. For example, the matrix metalloproteases have been extensively studied with many showing significant upregulation in tumors (36). Matrix metalloprotease inhibitors that were developed showed promising preclinical results but ultimately yielded disappointing results in clinical trials. This was due, at least in part, to intricacies of their physiologic and pathophysiologic roles (37, 38). Consequently, attention has focused...
on other proteases such as the cysteine cathepsins, particularly cathepsin B, which has been shown to be upregulated in a wide range of cancers (6, 39). Furthermore, in conjunction with the functional studies in RIP1-Tag2 mice showing attenuated development, growth, invasion, and neovascularization of tumors (11), the case for examining their therapeutic targeting is compelling. Indeed, these researchers have now successfully shown the application of a broad-spectrum irreversible cathepsin inhibitor (JPM-OEt) in combination with chemotherapeutics to block tumor development in the RIP1-Tag2 model (15). However, JPM-OEt has independently been shown to possess poor bioavailability, accumulating in cathepsin-rich organs such as the pancreas, kidneys, and liver (40). Moreover, given that cathepsin B and cathepsin L are widely expressed, the application of broad-spectrum cell-permeable inhibitors may not be clinically appropriate. This hypothesis is supported by the fact that cathepsin L and cathepsin B double-knockout mice exhibit severe neural development deficiencies and die within a few weeks of birth, as a result of cardiomyopathy (41, 42). Consequently, as cathepsin S has limited expression in normal tissue, and knockout animals are viable, the specific targeting of cathepsin S should avoid these issues.

The inhibition of cathepsin S was previously considered a potential therapeutic option in autoimmune disease, focusing on development of compounds to block its intracellular role in antigen presentation (43, 44). However, as a result of close family

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**Fig. 6.** Fsn0503 blocks tumor progression in vivo. A, administration of Fsn0503 s.c. (10 mg/kg; arrows) resulted in significant attenuation of HCT116 xenograft growth in flanks of Balb/C mice (P < 0.0001). B, immunohistochemical analysis of sections from these tumors showed reduced endothelial cell infiltration as visualized by CD34 staining (arrows) in tumors treated with Fsn0503.
homology, efforts to develop specific small molecule inhibitors towards cathepsin S and indeed other cathepsins have been hindered by the inability to achieve selectivity (45). Furthermore, side effects and toxicities may be further compounded by the accumulation of these inhibitors within the lysosomes (particularly in cathepsin-rich tissues such as the liver), where they reach concentrations exceeding selective inhibitors (27, 28). Given the potential pitfalls with such small molecule inhibitors, the application of antibodies to antagonize cathepsin S may offer significant therapeutic potential for cancer treatment. This approach allows the generation of a compound that is both specific towards cathepsin S, and also will not accumulate in lysosomes, due to endosomal degradation (46). Furthermore, as the pathologic cathepsin S is secreted into the tumor microenvironment, it is therefore amendable to antibody compounds, as shown in this investigation. However, the clinical utility of these initial findings with the antibody will need to be established through further investigation in preclinical and clinical models; particularly in combination with existing chemotherapeutic agents. This is necessary to ascertain the usefulness of cathepsin S as a cancer target and the applicability of therapeutic antibodies directed to it. This will need to be accompanied by detailed pathologic analysis of different tumor types in order to establish the most appropriate tumor classes to target. Indeed, as a result of the findings with the MDA-MB-231 cell line presented here, we are currently examining the levels of cathepsin S in breast carcinomas.

In conclusion, the use of an antibody that can selectively antagonize cathepsin S in the tumor milieu may help circumvent toxicity issues that are likely to arise with small molecule inhibitors. Collectively, our results exemplify the targeting of cathepsin S in tumors, such as colorectal carcinomas, using antibody-based therapeutic inhibitors to block tumor invasion and angiogenesis. We hypothesize that the development of drug regimes that combine chemotherapies with an anti-inflammatory/angiogenic agent, such as the antibody described here, could lead to improved clinical outcomes in aggressive and metastatic tumors.

Disclosure of Potential Conflicts of Interest

J. Johnston and C. Scott are consultants to Fusion Antibodies, Ltd. R. Burden, J. Johnston, and C. Scott have ownership interests in Fusion Antibodies, Ltd.

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