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Research paper

Fsn0503h antibody-mediated blockade of cathepsin S as a potential therapeutic strategy for the treatment of solid tumors

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ABSTRACT

Degradation of extracellular matrix components is a key step in tumor progression, facilitating invasion, angiogenesis, and metastasis. The lysosomal cysteine protease cathepsin S (Cat-S) is a prominent player in this process. We evaluated the antitumor activity of Fsn0503h, the first Cat-S–antagonistic humanized monoclonal antibody, in a panel of cancer cell lines and in human colon carcinoma xenografts. Cat-S was expressed in 11 out of 36 solid tumor-derived cell lines. Fsn0503h significantly reduced the invasive capacity of all Cat-S–expressing cell lines *in vitro*. This was confirmed by the Cat-S small-molecule inhibitor Z-FL-COCHO, validating the importance of this protease in tumor cell invasiveness. Interestingly, Fsn0503h displayed antiproliferative effects in Cat-S positive and some Cat-S–negative cell lines. We provide the first demonstration of *in vivo* activity of Fsn0503h against a colorectal tumor xenograft model, with a 10 mg/kg three times a week intravenous schedule being optimal. In conclusion, Fsn0503h not only inhibited the invasiveness of cancer cells *in vitro*, but also exerted antitumor effects both *in vitro* and *in vivo*. These findings validate Cat-S as a therapeutic target, and support the development of Fsn0503h for the therapy of solid tumors.

1. Introduction

Cathepsin S (Cat-S) is a lysosomal cysteine protease of the papain superfamily. Ten other members of this family have been identified in the human genome (cathepsin B, C, H, F, K, L, O, V, W and X), in a variety of cell types [1–3]. The B, L and S enzymes have been extensively studied in the context of cancer, and their role in tumor progression as well as their intrinsic limitations as therapeutic targets are well understood. Cathepsin L is considered to possess tumor suppressor properties [4]. The widespread tissue expression of cathepsin B [5] suggests that its inhibition would generate off-target toxicity concerns. Contrary to most cathepsins which are only active in acidic environments, Cat-S is active in both acidic and neutral conditions [6]. It is predominantly expressed in antigen-presenting cells such as macrophages, dendritic cells, and B lymphocytes [2,6], playing a key role in initiating antigen presentation to CD4+T-cells through intracellular degradation of the invariant peptide chain associated with the major histocompatibility complex class II [1,7,8]. There is also evidence that Cat-S is involved in the regulation of several pathological processes, such as obesity [9,10], Alzheimer's disease [11,12], bronchial asthma [13], cardiovascular disease [10] and diabetes [14,15].

Cat-S is overexpressed in several tumor types (colon, hepatocellular, prostate, melanoma, lung, ovarian, brain, pancreatic, head and neck, skin and breast), and expression levels correlate with

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ANOVA, analysis of variance; Cat-S, cathepsin S; DMSO, dimethyl sulfoxide; ECM, extracellular matrix; E_{max}, maximum effect; EMT, epithelial-mesenchymal transition; FCS, fetal calf serum; GI_{50}, growth inhibition concentration 50%; INF-γ, interferon gamma; IP, intraperitoneal; IV, intravenous; PBS, phosphate-buffered saline; SC, subcutaneous; SEM, standard error of the mean; SNK, Student-Newman-Keuls.

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clinical aggressiveness [16–23]. There is increasing evidence that Cat-S is secreted into the tumor environment causing degradation of extracellular matrix (ECM) components (laminin, fibronectin, elastin and collagen), thereby facilitating invasion, angiogenesis and metastasis during tumor progression [19,24,25].

The anti-Cat-S mouse-derived monoclonal antibody Fsn0503 (1E11) presents high specificity for Cat-S over the B, K and L proteases [26]. It also blocks human Cat-S cleavage of ECM proteins, resulting in decreased cancer cell invasion [25–28]. In addition, Fsn0503h and irinotecan showed synergistic effects on tumor growth in human colorectal carcinoma xenografts [28]. More recently, the first humanized Fsn0503-derived monoclonal antibody, Fsn0503h, has been demonstrated to bind Cat-S and negatively impact the viability of pancreatic and colorectal cancer cells by mediating antibody-dependent cellular cytotoxicity (ADCC) *in vitro* [29].

The current study was prompted by the limited data available on the activity of FSN0503h. We evaluated the potential antitumor application of Fsn0503h on a broad panel of human solid tumor-derived cell lines, selecting the most sensitive cell line to perform further *in vivo* experiments to assess different doses and dosing schedules in order to optimize clinical administration protocols.

2. Materials and methods

2.1. Reagents, cell lines, and antibodies

All reagents were obtained from Sigma–Aldrich (St Louis, MO, USA) unless otherwise specified. Cells lines (Table 1) were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA) and maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; GIBCO, #2614007), 2 mM glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified 5% CO_2 atmosphere. Cell lines were checked regularly for Mycoplasma infection using the MycoProbe Mycoplasma Detection Kit (R&D Systems) according to the manufacturer's instructions. IFN-γ was used to induce Cat-S expression.

The mouse-derived anti-cathepsin S monoclonal antibody Fsn0503 (1E11) and the humanized monoclonal antibody (Fsn0503h) were provided by Fusion Antibodies Ltd. [26–29]. The β-actin, anti-rabbit and anti-mouse HRP antibodies were purchased from Cell Signaling Technology. The Cat-S inhibitor Z-FL-COCHO (Calbiochem, Merck KGaA) was diluted in DMSO (11 mM) and stored at −20 °C.

Table 1
Cell lines, characteristics and Cat-S expression using the Fsn0503 (1E11) antibody.

Tumor type	Cell line	Comments	Cat-S
Breast	MDA-MB-361		+
Breast	MDA-MB-231		–
Breast	SKBR3		–
Breast	MCF7	Parental cell line	–
Breast	MCF7-shWhisp	From MCF7, EMT model with sh WISP2 [31]	–
Glioma	U87MG		+
Glioma	U251		–
Gioma	SF268		–
Hepatocarcinoma	Hep3B		–
Hepatocarcinoma	HepG2		–
Hepatocarcinoma	SK- HEP1		–
Hepatocarcinoma	SK-Suni	From SK-HEP1, acquired resistance to sunitinib® [35]	–
Hepatocarcinoma	SK-Sora	From SK-HEP1, acquired resistance to sorafenib® [35]	–
Cholangiocarcinoma	HuCCT1		+
Cholangiocarcinoma	MZ-CHA1		–
Cholangiocarcinoma	MZ-CHA2		–
Cholangiocarcinoma	Oz		–
Colon	Colo-205	Parental cell line	+
Colon	Colo-205R	From Colo205, EMT model with acquired resistance to PKC inhibitors [32]	–
Colon	DLD-1-TR21	Parental cell line	–
Colon	DLD-SNAIL	From the DLD-1TR21, EMT model with *SNAIL* inducible expression [33]	–
Prostate	DU145		–
Prostate	PC3		+
Prostate	LNCap		+
Renal	CaKi-1		+
Renal	CaKi-2		+
Renal	786-0		+[a]
Pancreatic	CaPan-1		+
Pancreatic	MIAPaCa2		–
Pancreatic	Panc-1		+
Ovarian	Igrov-1		–
Ovarian	OVCAR3		–
Ovarian	1A9		–
Head and neck	SQ20B		–
Head and neck	Hep2		–
Head and Neck	SCC61		+

[a] 786-0 cells only expressed Cat-S after 24-h pretreatment with 50 ng/ml INF-γ.

2.2. Western immunoblotting

Cells were lysed in buffer containing 50 mM HEPES (pH 7.6), 150 mM NaCl, 1% Triton X-100, 2 mM sodium vanadate, 100 mM NaF, and 0.4 mg/ml phenylmethylsulfonyl fluoride. Proteins (30 μg/lane) were resolved by SDS-PAGE and transferred to Hybond-ECL membranes (Invitrogen). Membranes were blocked with 5% milk in 0.05% Tween-20/PBS, incubated with the primary anti-Cat-S antibody overnight (murine Fsn0503; 1:500), then washed and incubated with the secondary HRP-linked antibody (1:1000). β-actin (1:1000) was used as a loading control. Bands were visualized with the enhanced chemiluminescence Western blotting detection system (BioRad).

2.3. Cell migration and invasion assays

Invasiveness was measured using Matrigel invasion chambers (24-well, BD Biosciences). Membranes (8 μm) were coated with Matrigel (50 μg, BD Biosciences) and placed in the lower chamber containing 0.6 ml of RPMI 1640 medium with 20% FBS as chemoattractant. 10^5 cells were seeded on the inserts suspended in 0.3 ml of serum-free RPMI. After incubation for 24 h with or without Fsn0503h or Z-FL-COCHO, the upper surface of the filter was scraped to remove non-invasive cells. Invasive cells were fixed and stained with a Diff Quik Detection Kit (Dade Behring). The average number of invading cells per field was assessed by counting 9 random fields under a light microscope (400x).

2.4. MTT assay

Cells were seeded in 96-well culture plates at 2×10^3 cells/well. After 24 h culture they were treated for 48 h with Fsn0503h (0.03–4 μM), to determine the growth inhibition concentration 50% (GI_{50}). Antiproliferative effects of 250 nM Fsn0503h were also compared with 250 nM Z-FL-COCHO. After treatment, cells were incubated with 0.4 mg/ml MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) for 4 h. Supernatant was discarded, the cell pellet was resuspended in 0.1 ml DMSO, and absorbance was measured at 560 nm using a microplate reader (Thermo