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Review Article

Biochemical and structural insights into mesotrypsin: an unusual human trypsin

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Abstract: Thirty five years ago mesotrypsin was first isolated from the human pancreas. It was described as a minor trypsin isoform with the remarkable property of near total resistance to biological trypsin inhibitors. Another unusual feature of mesotrypsin was discovered later, when it was found that mesotrypsin has defective affinity toward many protein substrates of other trypsins. As the younger sibling of the two major trypsins secreted by the pancreas, cationic and the anionic trypsin, it has been speculated to represent an evolutionary waste with no apparent function. We know now that mesotrypsin is functionally very different from the other trypsins, with novel substrate specificity that hints at distinct physiological functions. Recently, evidence has begun to emerge implicating mesotrypsin in direct involvement in cancer progression. This review will explore the biochemical characteristics of mesotrypsin and structural insights into its specificity, function, and inhibition.

Keywords: Trypsin, mesotrypsin, serine protease, protease inhibitors, protein crystallography, substrate specificity, cancer progression

Introduction

Proteases are encoded by roughly 3% of all genes in organisms ranging from bacteria to plants to humans [1]. They are universally distributed throughout the human body and play essential roles in many important physiological and pathological functions. One of the most abundant mechanistic classes of proteases is the serine protease group, named for the key nucleophilic serine involved in catalysis. Trypsins are a group of serine proteases that are produced and secreted as zymogens by the pancreas and activated by enteropeptidase cleavage in the duodenum, where they act as major digestive enzymes [2].

Due to the potential for proteolytic tissue damage, elaborate mechanisms have evolved to regulate proteases at multiple levels. One mechanism involves complexation of proteases with endogenous protein protease inhibitors. Regulation of the protease/inhibitor balance is crucial, and the upset of this balance is in evidence in numerous pathological conditions, including forms of cancer, emphysema, cystic fibrosis, chronic obstructive pulmonary disease, acute respiratory distress syndrome, asthma, cirrhosis, thrombosis, dementia, pancreatitis, and psoriasis [3-8]. One major group of endogenous inhibitors of trypsins and other serine proteases are the 'canonical' inhibitors, named for a protease-binding loop of highly characteristic backbone conformation [9-12]. A canonical inhibitor fulfills the paradoxical function of binding to trypsin or another serine protease in a substrate-like manner, and yet acting as an inhibitor rather than a substrate [11, 13]. A particularly impressive example is the interaction of the canonical inhibitor bovine pancreatic trypsin inhibitor (BPTI) with bovine trypsin, where at neutral pH, $K_i \approx 5 \times 10^{-14}$ M [14, 15], and $k_{cat} \approx 8 \times 10^{-10}$ s^{-1} [16, 17].

Human mesotrypsin, encoded by the *PRSS3* gene, has been described as a defective human trypsin due to its compromised ability to cleave protein substrates, and its physiological role has been a mystery [18-20]. While mesotrypsin has long been known to be resistant to inhibi-

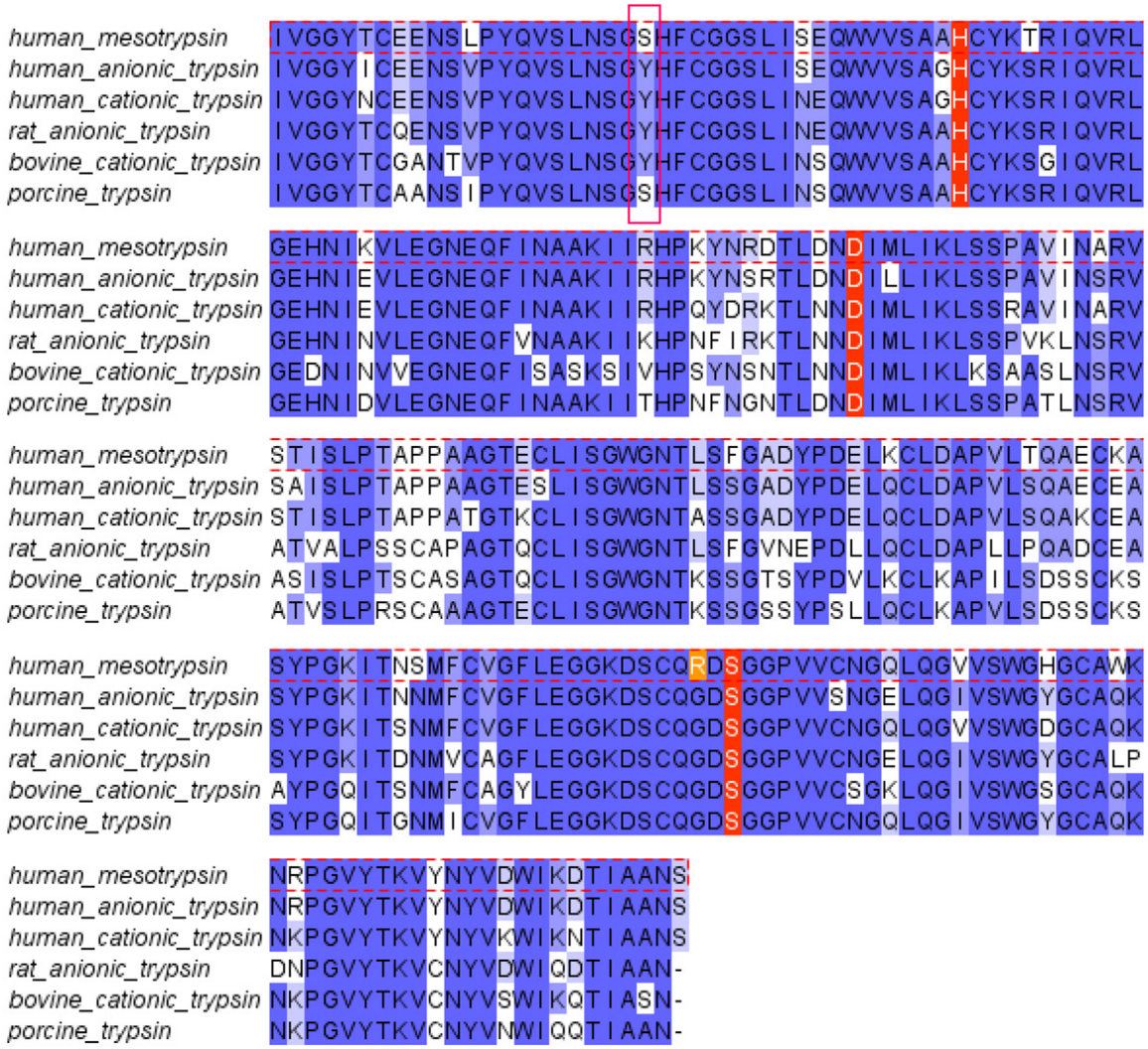

Figure 1. Sequence alignment of human trypsins. Human mesotrypsin displays a high degree of conservation with other mammalian trypsins. Two important differences are the substitution of Arg for the conserved Gly-193 (highlighted in orange) and the substitution of Tyr-39 with Ser (pink box).

tion by protein protease inhibitors [18, 19, 21, 22], in 2003 it was discovered that mesotrypsin possesses an extraordinary catalytic capability for hydrolyzing the reactive sites of canonical trypsin inhibitors SPINK1 and soybean trypsin inhibitor [19]. In the past five years, evidence has continued to mount suggesting that mesotrypsin may have evolved very specifically to carry out the unique task of proteolyzing canonical inhibitors as substrates [23-25]. Here, we will describe the structural and enzymatic features of mesotrypsin that account for this unusual catalytic capability. We will also explore emerging evidence for its role in cancer, and consider the potential for development of potent and selective mesotrypsin inhibitors.

Human trypsin genes and proteins

Two major human trypsinogens, trypsinogen 1 and trypsinogen 2, were first purified in 1969 [26]. Trypsinogen 1 is the most abundant, accounting for ~13% of proteins in human pancreatic juice, while trypsinogen 2 accounts for ~6% of proteins in human pancreatic juice [27]. A third, minor trypsinogen isoform, originally referred to as “zymogen X”, was isolated by Rinderknecht et al. in 1979 [27]. It occurs in very low concentrations and probably accounts for <0.5% of proteins in human pancreatic juice. Human trypsinogens I, II and “zymogen X” were renamed on the basis of their relative isoelectric points as cationic, anionic and meso-

Table 1. Crystal structures of mesotrypsin complexes

PDB ID	Enzyme	Inhibitor	Resolution (Å)	Reference
1H4W	mesotrypsin	benzamidine	1.70	Katona *et al.* [22]
1R9P	mesotrypsin-S195A	BPTI	1.40	Salameh *et al.* [43]
3L33	mesotrypsin-S195A	APPI	2.48	Salameh *et al.* [25]
3L3T	mesotrypsin-S195A	APPI-R15K	2.38	Salameh *et al.* [25]
3P92	mesotrypsin-S195A	BPTI-K15R/R17G	1.60	Salameh *et al.* [42]
3P95	mesotrypsin-S195A	BPTI-K15R/R17D	1.30	Salameh *et al.* [42]
4DG4	mesotrypsin-S39Y/S195A	BPTI	1.40	Salameh *et al.* [24]

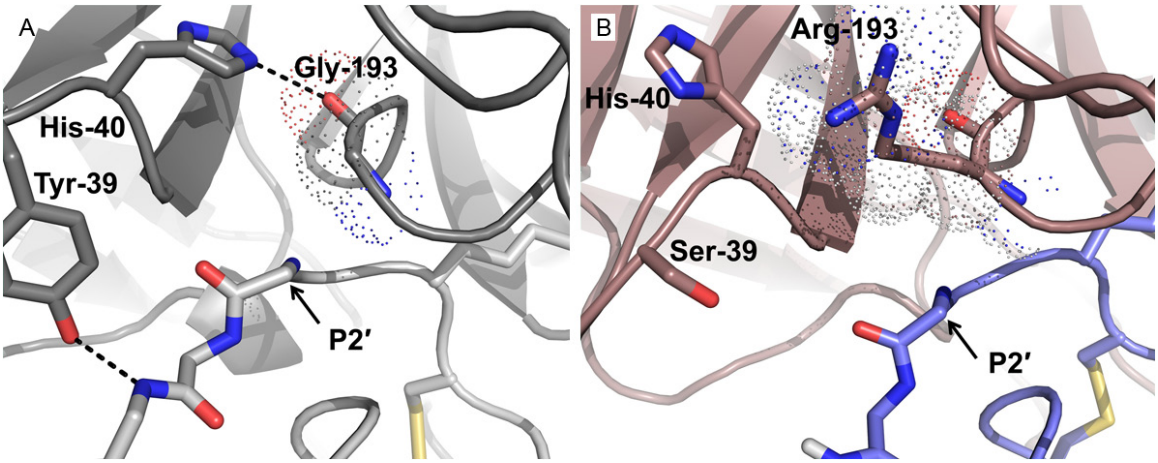

Figure 2. Unique structural features of the mesotrypsin active site. Illustration of major structural differences between cationic trypsin and mesotrypsin. A. In the cationic trypsin complex with BPTI (PDB: 1TRN), the a highly conserved Gly-193 forms an H-bond with His-40 and Tyr-39 forms an H-bond with the main chain amide nitrogen of the P_4' residue of BPTI. B. By contrast, the mesotrypsin complex with BPTI (PDB: 2R9P) features fewer intermolecular H-bonds, as well as the potential for steric and/or electrostatic repulsion by Arg-193 of an inhibitor or substrate possessing a bulky side chain at the P_2' position. In both panels, the Arg-17 P_2' side chain of BPTI has been omitted for clarity.

trypsinogen, respectively [18, 27]. The three human trypsinogens are encoded by different genes; those for cationic trypsinogen (*PRSS1*) and anionic trypsinogen (*PRSS2*) are located at proximal loci on chromosome 7q35, while the gene encoding mesotrypsinogen (*PRSS3*) is found on chromosome 9p13 [28].

Differentially-spliced forms of mesotrypsinogen, transcribed from alternative promoters, are expressed in different tissues [29-31]. Trypsinogen 4, which may utilize an unconventional CUG translation initiation codon [30], is highly expressed in brain tissue [32, 33], and expressed at lower levels in many tissues and some tumors [29, 34-39]. Trypsinogen 5, the most recently identified isoform, appears to have more restricted expression limited primarily to brain, intestine, uterus, and keratinocytes [29]. The multiple zymogen forms differ only at the N-terminus encoded by exon 1, such that processing of any of the isoforms by removal of the prodomain results in active mesotrypsin of identical amino acid sequence [31]. Trypsinogens 4 and 5 lack recognizable signal sequences, but a feature-based algorithm for predicting non-classical and leaderless secreted proteins [40] scores these zymogens as likely candidates for nonclassical secretion. There is some evidence for processing of the prodomain of trypsinogen 4 and deposition of activated mesotrypsin in the extracellular neuronal matrix of the brain [32], and likewise for activation of trypsinogens 4 and 5 by enteropeptidase in the granular layer of the epidermis [29]. Although active mesotrypsin protein has not been directly detected in tumors, mesotrypsin activity has been implicated in tumor progression by studies using *PRSS3* silencing or mesotrypsin inhibition in cancer models, as

Table 2. Comparison of trypsin and mesotrypsin inhibition by canonical serine protease inhibitors

Enzyme	Inhibitor	K_i (M)	Reference
bovine trypsin	bovine pancreatic trypsin inhibitor (BPTI)	6×10^{-14}	[14, 15]
bovine trypsin	soybean trypsin inhibitor (SBTI)	1×10^{-11}	[68]
porcine trypsin	SPINK1 (human)	3×10^{-11}	[15]
bovine trypsin	Bowman-Birk inhibitor (soybean)	8×10^{-10}	[69]
bovine trypsin	Curcurbita maxima trypsin inhibitor I	2×10^{-12}	[70]
bovine trypsin	Schistocerca gregaria trypsin inhibitor	3×10^{-12}	[71]
bovine trypsin	sunflower trypsin inhibitor	1×10^{-10}	[72]
porcine trypsin	bovine pancreatic trypsin inhibitor (BPTI)	1×10^{-11}	[73]
porcine trypsin	APPI (human)	1×10^{-10}	[73]
human cationic trypsin	bovine pancreatic trypsin inhibitor (BPTI)	2×10^{-11}	[43]
human cationic trypsin	APPI (human)	1.8×10^{-10}	[23]
human mesotrypsin	bovine pancreatic trypsin inhibitor (BPTI)	1.4×10^{-5}	[25, 43]
human mesotrypsin	soybean trypsin inhibitor (SBTI)	4.2×10^{-7}	[22]
human mesotrypsin	SPINK1 (human)	1.5×10^{-6}	[19]
human mesotrypsin	APPI (human)	1.4×10^{-7}	[23, 43]

described below, under "Role of mesotrypsin in cancer".

Sequence and structural differences between mesotrypsin and other human trypsins

The three trypsins belong to the chymotrypsin superfamily of serine endopeptidases that are characterized by the catalytic triad His-57, Asp-102 and Ser-195 (chymotrypsinogen numbering) [41]. They are all stabilized by Ca^{2+} as a ligand, and strictly cleave peptide bonds after Arg or Lys optimally at pH around 8.0 [18]. Mesotrypsin shows high sequence homology with the major digestive trypsins (**Figure 1**). Cationic trypsin (Uniprot: P07477) and anionic trypsin (Uniprot: P07478) are 96% identical, while mesotrypsin (Uniprot: P35030) shares 87.8% and 88.7% identity with cationic and anionic trypsin, respectively. Mesotrypsin exhibits unique sequence and structural features that contribute to distinct specificity and functional properties, most notably Arg-193, which is a highly conserved glycine residue in most other serine proteases.

Structural studies of mesotrypsin (**Table 1**) have revealed that Arg-193 contributes to an atypical clustering of positive electrostatic surface potential on the primed side of the substrate binding cleft (which interacts with substrate residues C-terminal to the cleavage site), in the vicinity of the S_2' subsite [22]. Co-crystal structures with several polypeptide trypsin inhibitors, along with mutagenesis studies, have demonstrated steric and in some cases electrostatic repulsion between Arg-193 and the P_2' residue of a bound substrate or inhibitor (**Figure 2**) [25, 42, 43]. The structures reveal that Arg-193 must undergo considerable conformational rearrangements to allow binding of substrates or inhibitors with bulky P_2' residues, while analysis of the kinetics of proteolysis show that this reduces affinity toward peptide inhibitors or substrates by several orders of magnitude compared to other trypsins [23, 25, 42, 43]. As a result, mesotrypsin is uniquely defiant to inhibition by many polypeptide serine protease inhibitors (**Table 2**) [18, 19, 21, 22], and is also inefficient in the cleavage of many tryptic sites within protein substrates, for example in the activation of other pancreatic zymogens [18, 19]. Mutation of mesotrypsin Arg-193 to Gly, as is found at this position in most other serine proteases, has been reported to restore sensitivity to inhibition by canonical trypsin inhibitors [19].

Another relatively conserved residue near the trypsin active site, Tyr-39, is substituted by Ser-39 in mesotrypsin (**Figure 1**). This particular residue was investigated recently to analyze its role in interactions with polypeptide trypsin inhibitors [24]. Tyr-39, but not Ser-39, forms a hydrogen bond with the main chain amide nitrogen of the P_4' residue of a bound protease

Table 3. Comparison of kinetic constants for proteolysis of specific substrates by mesotrypsin and other serine proteases

Enzyme	Substrate	K_m (M)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}s^{-1}$)	Reference
mesotrypsin	APPI	1.4×10^{-7}	0.042	3.0×10^{5}	[23]
mesotrypsin	SBTI	1.5×10^{-6}	~0.16†	~1.0×10^{5}†	[19]
cationic trypsin	chymotrypsinogen A	2.1×10^{-5}	2.3	1.1×10^{5}	[74]
enteropeptidase	cationic trypsinogen	1.4×10^{-6}	35	2.5×10^{7}	[75]
t-PA	Lys-plasminogen	7.6×10^{-6}	0.2	2.9×10^{4}	[76]
u-PA	Lys-plasminogen	5.4×10^{-6}	1.7	3.1×10^{5}	[77]

†Values are approximations from data shown in reference [19], based on an apparent initial rate of hydrolysis of ~0.04 s^{-1} in a reaction containing 500 nM SBTI, a concentration 3-fold below below the K_m as estimated by the reported K_i.

inhibitor (**Figure 2**). The presence of Ser-39 relative to Tyr-39 results in a 4- to 13-fold poorer binding affinity with the protease inhibitors examined. Thus, we concluded that the presence of Ser-39 in mesotrypsin, and corresponding absence of a single H-bond to the inhibitor backbone, makes a small but significant functional contribution to the resistance of mesotrypsin to inhibition [24]. Further studies may explore whether additional residue substitutions found in mesotrypsin contribute further to its unique inhibitor resistance and specificity.

Substrates and physiological function of mesotrypsin

Serine proteases in general carry out vital responsibilities relating to all aspects of human physiology, including roles in food digestion, reproduction, blood coagulation, signal transduction, apoptosis, and the immune response [44]. The identification of genes for many serine proteases of yet-unknown function suggests that additional roles remain to be uncovered [1]. Given its low concentration in the digestive juice and its apparent ectopic expression across a range of diverse tissues, the physiological function of mesotrypsin has remained enigmatic, but recent biochemical studies offer suggestions and clues. Compared to known physiological protein substrates of other trypsins, mesotrypsin is a poor enzyme. It fails to activate pancreatic zymogens, and also shows reduced capacity to degrade trypsinogens [19]. It is also substantially compromised in its ability to cleave protease activated receptors (PARs) [45-47]. Intriguingly, however, mesotrypsin displays enhanced catalytic activity, relative to other trypsins, toward one class of protein substrates: the canonical inhibitors of serine proteases [19, 23, 25, 43].

Canonical inhibitors ordinarily function as extremely tight-binding but proteolysis resistant substrate mimics for their cognate proteases, via a standard mechanism in which the peptide bond targeted for cleavage is thermodynamically stable [11, 13, 48, 49]. Due to intramolecular stabilization that maintains the inhibitory binding loop in a canonical conformation, perfectly prearranged for insertion into the protease active site [9, 10, 12], affinity is typically 5-7 orders of magnitude tighter than the comparable interaction with an unstructured peptide substrate [50, 51]. Rather than being cleaved and released as would be a normal substrate, the canonical inhibitor lingers in the protease active site, with the reverse (peptide religation) reaction outpacing the forward (cleavage) reaction [11, 48].

Mesotrypsin breaks this pattern—the effect of Arg-193, Ser-39, and perhaps additional residues unique to mesotrypsin results in a reduction in inhibitor affinity, from the picomolar range typical of protease/canonical inhibitor interactions to the micromolar range more typical of protease/substrate interactions (**Tables 2** and **3**). Unlike most other proteases, mesotrypsin appears to recognize its substrates in part via “conformational specificity”, such that presentation of a peptide substrate in the context of a stabilized canonical loop is essential for efficient substrate recognition [23]. Interestingly, the sequence and structural features of mesotrypsin that confer this binding specificity also dramatically enhance the rate with which mesotrypsin cleaves canonical inhibitors, bringing the kinetic constants K_m, k_{cat}, and the specificity constant k_{cat}/K_m in line with those of other proteases for their specific substrates (**Table 3**). The structural explanation for this gain of function appears to involve the

weakening of favorable interactions, and introduction of unfavorable interactions, between mesotrypsin and the primed-side residues of the canonical binding loop [24, 25, 43]. This results in expulsion of these residues from the active site upon inhibitor cleavage, disfavoring religation of the cleaved inhibitor.

Given these observations, we have asked whether some of the canonical serine protease inhibitors might be in fact physiological substrates of mesotrypsin. In the digestive system, one obvious potential use of mesotrypsin's unique digestive capability could be the inactivation and breakdown of protease inhibitors in the diet. Canonical inhibitors are widely distributed across the plant and animal kingdoms and are especially enriched in many plant food sources such as legumes, grains, and vegetables [11, 52]. The Sahin-Tóth laboratory first demonstrated the rapid hydrolysis of the reactive site bond of the canonical soybean trypsin inhibitor (SBTI) [19]; from their published data we have estimated the kinetic constants for this cleavage to resemble those of other protease/substrate interactions (**Table 3**).

In tissues and organs outside of the digestive system, mesotrypsin may be involved in the inactivation or clearance of some of the dozens of endogenous human canonical protease inhibitors. In search of potential mesotrypsin substrates produced in prostate cancer cells, we conducted an affinity-based proteomic screen to identify secreted trypsin inhibitors cleaved by mesotrypsin [23]. We found that the amyloid precursor protein (APP), which contains a protease inhibitor domain belonging to the I2 or Kunitz-BPTI family and is secreted as a physiological inhibitor of factor XIa (sAPP/protease nexin 2), is rapidly and selectively cleaved by mesotrypsin within the Kunitz protease inhibitor domain (APPI). Cleavage of APPI by mesotrypsin compromises its inhibition of other serine proteases, including cationic trypsin and factor XIa, by two orders of magnitude [23]. Considering that APP is a ubiquitously expressed transmembrane protein and is coexpressed with mesotrypsin in a number of tissues, it likely represents the first endogenous physiological substrate of mesotrypsin to be identified. Processing by mesotrypsin may regulate the protease inhibitory function of sAPP/protease nexin 2 *in vivo*, and may also modulate other activities of APP that involve the Kunitz domain. Further studies are needed to determine whether other endogenous human protease inhibitors may also be physiological substrates of mesotrypsin.

Role of mesotrypsin in cancer

While normal expression of the *PRSS3* gene encoding mesotrypsin is highest in pancreas and brain with very limited expression elsewhere [29, 53-55], we and others have found *PRSS3* to be transcriptionally upregulated with cancer progression in epithelial cancers including lung, colon, breast, pancreas, and prostate [35-38, 56]. The first report to offer mechanistic insight into the potential role of mesotrypsin in cancer progression was published in 2009 [37]. In this study, Hockla *et. al*. identified PRSS3 upregulation with malignant progression in a breast cancer cell line progression series. Whereas knockdown of PRSS3 by RNA interference approaches correlated closely with suppression of malignant growth in 3D cultures, treatment of cells with recombinant purified mesotrypsin enhanced the malignant growth phenotype [37]. Employing a proteomic screen of cleavage products from cell culture conditioned media, we identified the cell surface glycoprotein CD109 as a potential mesotrypsin substrate involved in driving malignancy; however it is not yet known whether the shedding of CD109 is directly catalyzed or indirectly mediated by mesotrypsin [37].

Another study, by Jiang *et al.,* described the involvement of PRSS3 in the progression of pancreatic cancer [38]. They found that PRSS3 expression correlated with metastasis and poor survival in pancreatic cancer patients. Using several cell culture and mouse xenograft models of pancreatic cancer, they found that over-expression of PRSS3 promoted invasion and proliferation of pancreatic cancer cells in vitro, as well as metastasis *in vivo*, while suppression of PRSS3 expression reduced cell invasion and delayed progression to metastasis [38]. The effects of mesotrypsin appeared to be mediated at least in part by upregulation of VEGF expression via the PAR1-mediated ERK pathway [38].

Most recently, the involvement of mesotrypsin in prostate cancer progression has been established by the Radisky group [36]. After observing that PRSS3 expression was upregulated in

metastatic prostate tumors, and that its expression in primary tumors was associated with recurrence, we used a mouse orthotopic model with bioluminescent imaging to show that PRSS3/mesotrypsin expression is critical for prostate cancer metastasis. Silencing of PRSS3 inhibited anchorage-independent growth of prostate cancer cells in soft agar assays, and suppressed invasiveness in Matrigel trans-well assays and three-dimensional (3D) cell culture models. In addition, treatment with recombinant mesotrypsin directly promoted an invasive cellular phenotype in prostate cancer cells, a specific effect that required the proteolytic activity of mesotrypsin; neither cationic trypsin nor a mesotrypsin mutant lacking activity could similarly drive this invasive phenotype [36].

Engineering polypeptide inhibitors for mesotrypsin

Protein therapeutics represent a growing segment of the drug discovery field, accounting for a quarter of recent new drug approvals [57]. Several polypeptide serine protease inhibitors, some already clinically approved [58, 59] or under study as investigational drugs, show high solubility, low toxicity, limited antigenicity, stability to digestion, and in some cases even retention of activity after oral administration [60-64], characteristics indicative of high potential as protein therapeutics. Since mesotrypsin has been implicated in progression of several different cancers as discussed above in "Role of mesotrypsin in cancer", we hypothesize that potent and selective inhibitors of mesotrypsin could potentially offer novel therapeutics. However, targeting mesotrypsin presents special challenges: mesotrypsin is resistant to inhibition by many polypeptide serine protease inhibitors, and possesses enhanced catalytic capability for hydrolyzing some inhibitors as highly specific substrates. A further challenge in the development of therapeutic mesotrypsin inhibitors is likely to be the identification of sufficiently selective inhibitors, since mesotrypsin belongs to a large family of closely structurally related trypsin-like proteases. However, by taking advantage of the unique active site features of mesotrypsin, it may be possible to develop novel and selective, potentially therapeutic, inhibitors.

Natural polypeptide inhibitors of the Kunitz-BPTI family present feasible leads for the development of a therapeutic mesotrypsin inhibitor, since members of this family include several established and investigational drugs targeting other serine proteases [59, 60, 63-67]. The Kunitz domain scaffold is in general highly stable; however, mesotrypsin possesses highly enhanced catalytic activity toward some Kunitz domain inhibitors, which may represent physiological substrates rather than inhibitors as discussed above in "Substrates and physiological function of mesotrypsin" [23]. Therefore, it will be critical that we select or engineer a scaffold possessing adequate resistance to mesotrypsin degradation. The stability of Kunitz inhibitors toward mesotrypsin varies dramatically; for example we have shown that bovine pancreatic trypsin inhibitor (BPTI) is 300 times more stable to mesotrypsin proteolysis than is APPI [25]. BPTI and APPI share the characteristic Kunitz domain fold, in which the binding loop makes the majority of close contacts with the inhibited enzyme. This loop is supported by a compact scaffold with a three dimensional structure maintained by hydrophobic packing and three disulfide bonds. BPTI and APPI differ only at the P_1 and P_2' residues within the binding loop, and share 45% amino acid identity throughout the scaffold. Mesotrypsin affinity toward the inhibitors is nearly exclusively modulated by the binding loop sequence, as mutation at the P_1 and P_2' positions of BPTI creates an inhibitor with affinity essentially equal to that of APPI, and vice versa [25]. Surprisingly, these mutations confer minimal effects on cleavage rates; thus, the greater stability of BPTI to mesotrypsin hydrolysis is largely attributable to the scaffold, rather than to the binding loop sequence [25]. As a result, a scaffold can be selected for optimal proteolytic stability, and then the binding loop modified to confer optimal affinity and enhance selectivity.

In optimizing affinity, we have found the P_1 residue to be of particular importance, since Arg at this position (versus Lys) favors tighter binding by a factor of six [25]. The amino acid nature of the P_2' residue is also extremely important, as bulky and charged residues strongly disfavor binding, whereas acidic residues facilitate cleavage [42]. We have recently engineered a polypeptide inhibitor based upon the BPTI scaffold and incorporating mutations at the P_1 position (Lys-15 to Arg) and at the P_2' position (Arg-17 to Gly) [42]. This new inhibitor, designated BPTI-K15R/R17G, is a relatively stable, high

affinity mesotrypsin inhibitor with an equilibrium binding constant K_i of 5.9 nM, a >2000-fold improvement in affinity over native BPTI. This engineered inhibitor demonstrated high efficacy for inhibiting mesotrypsin in assays of breast cancer cell malignant growth and pancreatic cancer cell invasion [42]. It also suppressed prostate cancer cell invasion to a similar extent as did PRSS3 gene silencing [36]. Our work to date suggests that inhibition of mesotrypsin activity may provide a novel modality for treatment of cancer, although further improvements in inhibitor selectivity will be important before clinical potential can be realized.

Concluding remarks

Mesotrypsin is a very unusual enzyme, possessing active site steric and electrostatic features unique among human serine proteases. These distinctive active site features are responsible for the resistance of mesotrypsin to many polypeptide trypsin inhibitors, and are also responsible for the unique substrate specificity of mesotrypsin toward protein protease inhibitors. Based on the observation that other trypsins are unable to recapitulate the activity of mesotrypsin in driving the invasive phenotype of prostate cancer cells [36], we speculate that these same physical and chemical features are critical in the ability of mesotrypsin to drive cancer metastasis [36, 38]. Importantly, these same distinctive features may provide an opportunity to develop new inhibitors, complementary in shape and charge to the mesotrypsin active site, which will selectively target mesotrypsin for therapeutic purposes.

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Disclosure of conflict of interest

None.

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