New potential therapeutic applications of WX-UK1, as a specific and potent inhibitor of human trypsin-like proteases

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Abstract

WX-UK1 (the active metabolite of upamostat) was originally developed as an inhibitor of the serine protease urokinase (uPA) with a K_i ~1 uM. To identify more sensitive targets, we performed a bioinformatic analysis of the ~200 human trypsin-like serine proteases, many of which play crucial roles in homeostasis and disease. Among these we selected a subset for biochemical analysis based on an inspection of modelled 3D structures of WX-UK1:protease complexes and sequence alignment of binding site residues. Samples of the selected proteases were prepared and characterized for their binding to WX-UK1; enzymatically with respect to inhibition constant (K_i) and by surface plasmon resonance with respect to dissociation constant (k_d). We now report that WX-UK1 is a potent and specific inhibitor of five human serine proteases (trypsin-3, trypsin-2, trypsin-1, matriptase-1 and trypsin-6), with K_i's down to the low nanomolar range, 19 nM for trypsin-3. Several of these serine proteases are known to be associated with cancer progression and metastasis. As a compound with an established clinical safety profile, targeted use of upamostat in oncology and extension to non-oncology indications may be assessed.

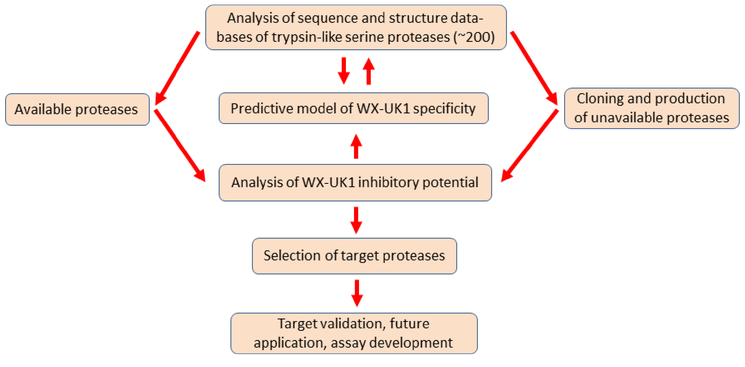
Introduction

Proteases are a vital part of life. They are involved in many physiological processes such as protein turnover /processing, digestion, blood coagulation and fibrinolysis, tissue remodeling and wound healing, fertilization, cell differentiation, programmed death and growth, cell signaling, signaling cascades via PAR's, immune response, tumor invasion and metastasis. Uncontrolled protease activity is associated with many pathophysiological conditions such as emphysema, stroke, viral infections, inflammation, arthritis cancer.

Of the total 1000 human peptidases, approximately 200 are serine proteases of the S1A chymotrypsin family with approximately 90 having trypsin-like specificity. The chymotrypsin-like S1A serine proteases are the most abundant family of peptidases in the human degradome (Page *et al*, 2008). The specificity of the chymotrypsin superfamily is determined largely by resides 189, 216 and 226 in the S1 pocket (Branden and Tooze, 1999).

Given the pleiotropic role of chymotrypsin-like proteases, specific inhibitors would be expected to have therapeutic value. Thus we have used our compound, WX-UK1, which was developed as an inhibitor to uPA to find other targets to which it may bind and provide potential therapeutic value.

Figure 1: Iterative process used to identify WX-UK1 targets



Methods

By a thorough analysis of the sequence database and available structural information of all human chymotrypsin-like serine proteases with trypsin-like specificity, a circular iterative process was carried out in which structure and sequence based criteria for WX-UK1 inhibition was tested by measuring WX-UK1 inhibition of selected protease. Based on the biochemical data, the database was repetitively reanalyzed to improve the selection criteria (Figure 1). This approach allowed for the identification of the structural features that predicted high affinity binding of WX-UK1 and focusing of our efforts on these potential targets.

Proteases not commercially available were produced from bacteria, yeast or higher eukaryotic cells. The identity, purity and activity of all proteases (including purchased), were verified by Mass spectroscopy and biochemical analysis. Enzymatic activity was characterized using standard Michaelis-Menten Kinetics.

Results

Application of the iterative process described in the methods resulted in a progressively smaller selection of potential high-affinity targets as shown in Figure 2. During this process, key structural components were elucidated as shown in Figure 3. Enzymatic characterization of the five proteases identified with Ki values ≤ 200nM is shown in Table 1, along with the residues in key positions. Two other proteases are still being characterized as potentially meeting this definition.

Figure 2: Iterative process used to identify WX-UK1 targets resulted in progressively focused selection of potential high-affinity targets

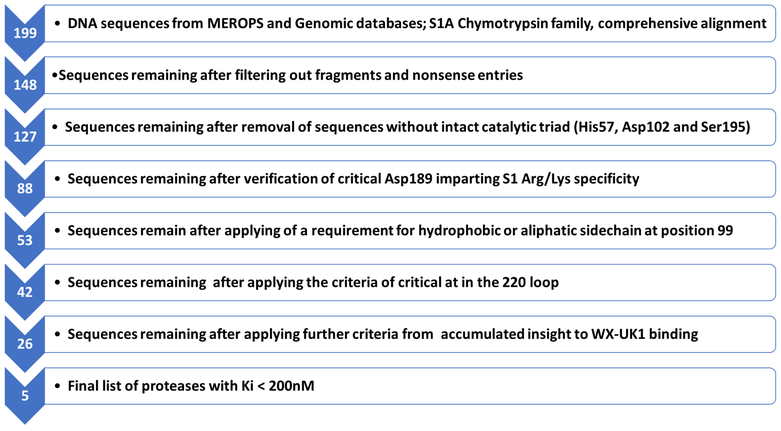


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Figure 3: Structural segments on the protease surface in and around the active site are primary predictive determinants of WX-UK1 binding (Bovine trypsin:WX-UK1, PDBID 1F92).

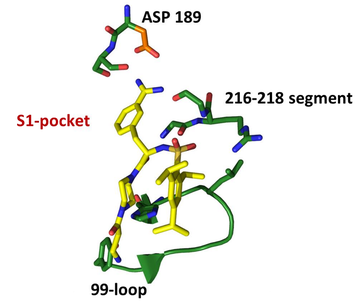

Table 1. Ki's for the five proteases identified thus far and residues in critical positions; Ki values are shown ± standard deviation and with the number of determinations (n)

Protease	Ki (μM)	97	99	216	217	218
uPA	0.90 ± 0.1 (3)	L	H	G	R	G
Human Trypsin-3	0.019 ± 0.004 (6)	D	L	G	H	G
Human Trypsin-2	0.075 ± 0.003 (6)	R	L	G	Y	G
Human Trypsin-6	0.10 ± 0.01 (4)	I	L	G	Y	G
Human Trypsin-1	0.19 ± 0.01 (3)	K	L	G	D	G
Human Matriptase-1	0.20 ± 0.01 (3)	F	F	G	D	G

Discussion

With the identification of these five new molecular targets, various disease indications may be proposed:

- As a low nanomolar inhibitor of human trypsin-3, WX-UK1 may find potential utility in the treatment of inflammatory digestive diseases, including irritable bowel syndrome, inflammatory bowel disease and pancreatitis, the latter for which there are currently no approved therapies
- As trypsin-3 is a poor prognosis indicator for pancreatic cancer (Jiang *et al*, 2010), expression may provide a patient selection biomarker for patients most appropriate for therapy with upamostat, the oral prodrug of WX-UK1
- As a human trypsin-2 inhibitor WX-UK1 could potentially be used in the treatment of inflammatory lung diseases, including acute respiratory distress syndrome, acute lung injury, α-1 antitrypsin deficiency and chronic obstructive pulmonary disease (COPD), the latter of which represents a major unmet medical need.

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