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Rhomboid protease inhibitors: Emerging tools and future therapeutics

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ABSTRACT

Rhomboid-family intramembrane serine proteases are evolutionarily widespread. Their functions in different organisms are gradually being uncovered and already suggest medical relevance for infectious diseases and cancer. In contrast to these advances, selective inhibitors that could serve as efficient tools for investigation of physiological functions of rhomboids, validation of their disease relevance or as templates for drug development are lacking. In this review I extract what is known about rhomboid protease mechanism and specificity, examine the currently used inhibitors, their mechanism of action and limitations, and conclude by proposing routes for future development of rhomboid protease inhibitors.

Contents

1. Introduction

The first members of the rhomboid-like superfamily were identified in *Drosophila* [1] as intramembrane serine proteases that activate the ligands of the epidermal growth factor (EGF) receptor [2]. Rhomboid protease homologs have since been found in nearly every sequenced genome spanning virtually all life forms, constituting the most widely occurring family of intramembrane proteases [3]. More recently, a number of related but proteolytically inactive members of the superfamily have been identified, such as iRhoms or Derlins [reviewed in [4]]. iRhoms are potential novel targets for TNFα [5–7] and ADAM17/EGFR [8–10] related pathologies, but their mode of action and druggability are unclear (for more discussion see the article by Lemberg and Adrain in this issue). The main mechanistic and structural model for the rhomboid superfamily have been rhomboid proteases.

Abbreviations: ABP, activity based probe; ADAM17, a disintegrin and metalloproteinase 17; cmk, chloromethylketone; cho, aldehyde; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FRET, Foerster resonance energy transfer; iRhom, inactive rhomboid homologue; PARL, presenilin-associated rhomboid-like; PD, Parkinson's disease; RHBDD, rhomboid domain-containing protein; RHBDL, rhomboid-like protein; TNFα, tumor necrosis factor α; TACE, TNFα-converting enzyme; TMD, transmembrane domain.

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Investigation in as distant fields as parasitology, cancer biology or microbiology has revealed exciting functions for rhomboid proteases in a variety of contexts. Although a large proportion of this territory is still unexplored, the few examples below already show that rhomboids are potential therapeutic targets. Investigation of biological roles of rhomboid proteases in multiple organisms would greatly benefit from the availability of selective rhomboid inhibitors, but these are currently not available. Here I review the current strategies to inhibit rhomboid proteases and summarize what the field has learned from them. I also review the current knowledge of rhomboid mechanism and specificity, and discuss its implications for future strategies of rhomboid inhibitor development. Let us first focus on examples and contexts where rhomboid proteases participate or can participate in disease-relevant processes and where selective rhomboid inhibitors could be employed for pharmacological applications.

2. Rhomboid proteases as potential future drug targets

2.1. Microbial rhomboid proteases

Rhomboid proteases are present in a number of protozoan parasites, such as *Plasmodium, Toxoplasma, Eimeria, Cryptosporidium, Theileria* and *Babesia* [11,12] including serious worldwide pathogens. Their functions have been addressed genetically and biochemically in *Plasmodium, Toxoplasma, Entamoeba* and *Trichomonas* so far. In the extracellular protozoan parasite *Trichomonas vaginalis*, which causes a sexually transmitted infection aggravating other disease conditions, rhomboid proteases TvROM1 and TvROM3 are active enzymes, and overexpression of the cell-surface localized TvROM1 enhances the association of the parasite with host cells and their lysis [13]. The extracellular parasite *Entamoeba histolytica* encodes one rhomboid protease EhROM1 that has been implicated in adhesion and phagocytosis [14,15]. The intracellular parasite *Plasmodium falciparum* causing malaria is probably the most serious medical burden of the above named infectious agents, affecting millions of people worldwide. The rhomboid proteases PfROM1 and PfROM4 of *P. falciparum* can cleave and shed several major surface adhesins of the parasite that are implicated in all stages of its life cycle [16]. Mutations in the transmembrane domain of adhesin EBA-175 inhibiting its cleavage by PfROM4 prevent the growth of the parasite [17], suggesting that PfROM4 is a potential therapeutic target. In *Plasmodium berghei*, a genetically tractable malaria model, PbROM1 deficiency did not compromise the infectivity or pathogenicity, but genes encoding rhomboids ROM4, 6, 7 and 8 were refractory to deletion, suggesting that these rhomboid proteases may be essential for the parasite, at least in its asexual blood stage [18]. Assuming that functions of the *P. berghei* rhomboids will be conserved in *P. falciparum*, there is an exciting prospect that inhibitors of several rhomboid proteases could have antimalarial activity.

Beyond protozoan parasites, the rhomboid protease RbdA from *Aspergillus fumigatus*, an opportunistic pathogenic mold encountered in immunocompromised individuals, is required for the adaptation of *A. fumigatus* to hypoxia during infection [19]. The RbdA deficient *A. fumigatus* is thus more sensitive to phagocytic killing, elicits weaker immune response and exhibits strongly attenuated virulence [19]. Since rhomboid proteases are widespread, it is likely that other disease-relevant functions in microbes will be discovered. In particular, relatively little is known about the functions of rhomboid proteases in bacteria [20–23] compared to how very widely distributed across prokaryotes rhomboids are [3,24].

2.2. Human rhomboid proteases

Rhomboid proteases are cardinal regulators of the EGF receptor signaling in *Drosophila*, but initially it seemed that their function in this pathway has not been conserved, because EGFR ligands in mammals are known to be activated by the ADAM family of membrane-bound metalloproteases [reviewed in Ref. [25,26]]. However, it is clear that the non-protease members of the rhomboid family of proteins called iRhoms control EGFR signaling in mammals by activating ADAM17 [8,10,27–29], the main EGFR ligand activating enzyme, and there is accumulating evidence that human rhomboid proteases may participate in fine-tuning of EGFR signaling [30–32]. This elevates the interest in understanding the role of mammalian rhomboid proteases in greater detail, and in development of their inhibitors as research tools.

There are four rhomboid proteases located in the secretory pathway of mammalian cells ('secretase' rhomboids RHBDL1-4) and one in the mitochondria (PARL) [33]. The best studied human secretase rhomboid is RHBDL2 (located at the plasma membrane [34]), probably because it is the only RHBDL that readily cleaves model rhomboid substrate Spitz [1] and has thus been amenable to enzymological and cell biological investigation. RHBDL2 is expressed mainly in epithelia [30], and it has been implicated in wound healing [35], endothelial angiogenesis [36], EGF receptor signaling [30,31], and possibly anoikis resistance [37], but loss-of-function animal experiments addressing these suggested functional hypotheses *in vivo* are lacking. Disease associations of RHBDL2 have not been reported apart from a recently found correlation between RHBDL2 mRNA levels and histological grade of breast cancer tumors [38].

The second best studied human secretase rhomboid is RHBDL4 (also known as RHBDD1), which is located in the endoplasmic reticulum. It has been implicated in membrane protein quality control [39], and shown to secrete TGFα [32,40] thus promoting the growth of colorectal cancer cells via activation of the EGF receptor [32]. The grade of colorectal cancer biopsies from patients and survival parameters correlated with RHBDL4 expression, and depletion of endogenous RHBDL4 from tumor cells suppressed proliferation *in vitro* and tumor growth *in vivo* [32], suggesting that inhibitors of RHBDL4 could have anti-tumor properties.

The remaining two human secretase rhomboids, RHBDL1 and 3 are the least characterized ones. They share about 49% sequence identity, and display overlapping but non-identical expression patterns, suggesting that they have distinct functions. RHBDL1 was the first human rhomboid protease gene to be identified [41]; it is expressed mainly in the brain and kidney [41] and localized to the Golgi apparatus [34]. RHBDL3 (also known as ventrhoid) is expressed in the developing neural ventral tube and in the brain [42], and is localized to the Golgi and plasma membrane [34]. It is also expressed in the developing pancreas under control of the neurogenin-3 transcription factor [43], but the significance of this observation for pancreas development and function is unclear. The expression level of RHBDL3 has been correlated with the chronological age and it is one of a few candidate markers of aging brain [44]. Both RHBDL1 and 3 bear all the sequence hallmarks of active rhomboid proteases, and RHBDL3 is able to bind an activity-based probe [45], suggesting that it has a functional active site. RHBDL1 and 3 might thus have a markedly different substrate specificity from the model rhomboid proteases (such as RHBDL2), but since no substrates of RHBDL1 and 3 have been identified so far, their molecular functions remain unknown.

The mitochondrial rhomboid PARL is the best characterized rhomboid protease in mammals. Mice deficient in PARL have a pronounced phenotype – muscle wasting and reduced lifespan – caused by increased apoptosis [46]. The basis for this effect is that PARL deficiency results in aberrant mitochondrial cristae, which