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Research article

Molecular design and structural optimization of potent peptide hydroxamate inhibitors to selectively target human ADAM metallopeptidase domain 17

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ABSTRACT

Human ADAMs (a disintegrin and metalloproteinases) have been established as an attractive therapeutic target of inflammatory disorders such as inflammatory bowel disease (IBD). The ADAM metallopeptidase domain 17 (ADAM17 or TACE) and its close relative ADAM10 are two of the most important ADAM members that share high conservation in sequence, structure and function, but exhibit subtle difference in regulation of downstream cell signaling events. Here, we described a systematic protocol that combined computational modeling and experimental assay to discover novel peptide hydroxamate derivatives as potent and selective inhibitors for ADAM17 over ADAM10. In the procedure, a virtual combinatorial library of peptide hydroxamate compounds was generated by exploiting intermolecular interactions involved in crystal and modeled structures. The library was examined in detail to identify few promising candidates with both high affinity to ADAM17 and low affinity to ADAM10, which were then tested in vitro with enzyme inhibition assay. Consequently, two peptide hydroxamates Hxm-Phe-Ser-Asn and Hxm-Phe-Arg-Gln were found to exhibit potent inhibition against ADAM17 (K_i = 92 and 47 nM, respectively) and strong selectivity for ADAM17 over ADAM10 (~7-fold and ~5-fold, S = 0.86 and 0.71, respectively). The structural basis and energetic property of ADAM17 and ADAM10 interactions with the designed inhibitors were also investigated systematically. It is found that the exquisite network of nonbonded interactions involving the side chains of peptide hydroxamates is primarily responsible for inhibitor selectivity, while the coordination interactions and hydrogen bonds formed by the hydroxamate moiety and backbone of peptide hydroxamates confer high affinity to inhibitor binding.

1. Introduction

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine that affects millions of people worldwide, and its peak onset is in persons 15–30 years of age (Loftus and Sandborn, 2002). The IBD comprises primarily two disorders: ulcerative colitis (UC) and Crohn's disease (CD). CD generally involves the ileum and colon, but it can affect any region of the intestine, often discontinuously; UC involves the rectum and may affect part of the colon or the entire colon in an uninterrupted pattern (Abraham and Cho, 2009). The hallmark of IBD is chronic, uncontrolled inflammation of the intestinal mucosa, which can affect any part of the gastrointestinal tract (Hanauer, 2006). Accumulating evidences suggest that the IBD results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host and a large number of cellular factors are involved in the pathological process, in which the tumor necrosis factor alpha (TNFα) is one of the most crucial mediators of this abnormal immune response (Magro and Portela, 2010), which has now become a well established target for IBD therapy (Ardizzone and Porro, 2005). The engineered monoclonal antibody Infliximab has largely revolutionized treatment of IBD and represents the first effective biologic therapy by targeting TNFα (Travassos and Cheifetz, 2005). Although the era of TNFα antibody therapy was a remarkable progress in combating IBD, many patients do not respond to the anti-TNFα treatment (Neurath, 2014). The effect of Infliximab is of limited duration, with the response lasting 2–3 months in most patients (Su et al., 2001). In addition, direct inhibition of TNFα may cause a variety of

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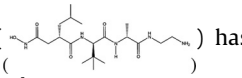
unexpected adverse effects such as morphea (Stewart et al., 2013) and central nervous system toxicity (Sand and Thomsen, 2013).

Several new therapeutic targets for IBD have been discovered over the past decade, including PPARγ (Dubuquoy et al., 2006), Jak/Stat (Coskun et al., 2013) and metalloproteinases (Naito and Yoshikawa, 2005). The TNFα-converting enzyme, also known as ADAM (a disintegrin and metalloproteinase) metallopeptidase domain 17 (ADAM17), releases transmembrane TNFα from cell surfaces of immune system to yield the soluble form of TNFα that acts as a compartmentalized or circulating cytokine to regulate complicated immune responses by providing direct signals required for full activation of effectors and regulatory T cells (Ślebioda and Kmieć, 2014). Although a number of proteases have been shown to process proTNFα, none do so with the efficiency of ADAM17. The ADAM17 is a membrane-bound enzyme that cleaves a large number of cell surface proteins including the proTNFα (Scheller et al., 2011). A series of orally bioavailable, selective and potent ADAM17 inhibitors are currently in clinical development, although none of them has been successfully applied into patients to date (Saftig and Reiss, 2011). These inhibitors effectively block ADAM17 mediated processing of proTNFα and can reduce soluble TNFα production by lipopolysaccharide stimulated whole blood by >95% (Newton et al., 2001). Thus, selective inhibition of ADAM17 has been raised as a promising therapeutics for IBD and other inflammatory diseases (Brynskov et al., 2002; Kirkegaard et al., 2004; Moss et al., 2008).

The ADAM10 is a close relative of ADAM17; both of them share high conservation in sequence, structure and function, but exhibit subtle difference in regulation of downstream cell signaling events (Duffy et al., 2011). Here, we performed rational design and structural optimization of peptide hydroxamate inhibitors with high potency against ADAM17 and strong selectivity for ADAM17 over ADAM10. Hydroxamate derivatives have been widely used as the metal coordination-based inhibitors of Zn^{2+}-containing metallopeptidases (Jani et al., 2005). In the procedure, the intermolecular interactions of ADAM17 and ADAM10 with peptide hydroxamate ligands were examined systematically based on crystal and modeled complex structures. The harvested knowledge were then used to derive a virtual combinatorial library of peptide hydroxamate compounds by optimizing molecular structures in order to achieve high affinity for ADAM17 and low affinity for ADAM10 simultaneously, from which few promising candidates were selected and tested in vitro with enzyme inhibition assay to substantiate the computational findings. The molecular mechanism of inhibitor selectivity between ADAM17 and ADAM10 was also investigated in detail by dissecting the structure basis and noncovalent property of inhibitor recognition by the two enzymes.

2. Materials and methods

2.1. Structure preparation

The crystal structure of ADAM17 catalytic domain complexed with its cognate inhibitor TAPI2 () has been solved at a high resolution of 1.9 Å via X-ray crystallography (Fig. 1A) (Mazzola et al., 2008), which was retrieved from the PDB database (PDB: 3EDZ) (Berman et al., 2000) and used as structure template to computationally construct the theoretical structure of ADAM10 catalytic domain. The primary sequences of ADAM17 (residues 223–470) and ADAM10 (residues 207–453) catalytic domains were curated from the UniProt database (Uniprot, 2010) under accession numbers P78536 and O14672 respectively, and then sequence alignment between them was carried out using

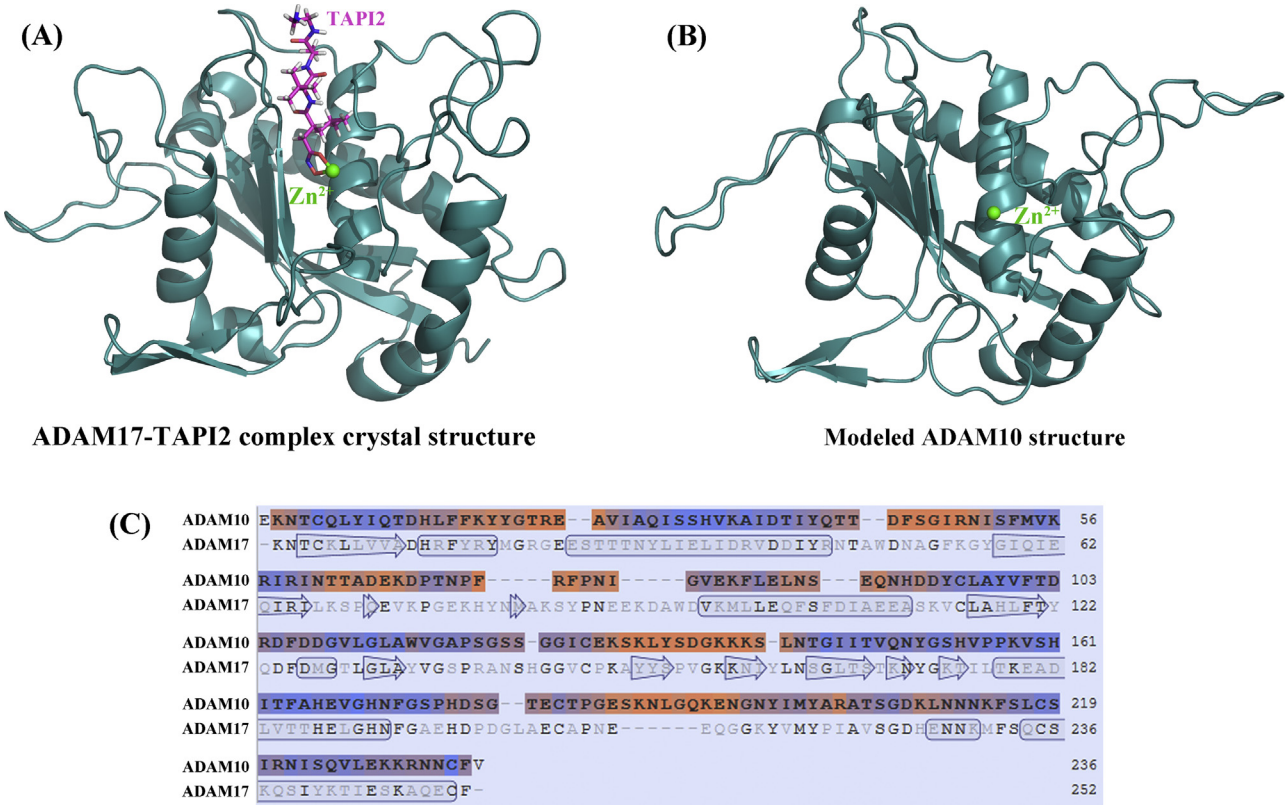


Fig. 1. (A) Crystal structure of ADAM17–TAPI2 complex (PDB: 3EDZ). (B) Computationally modeled ADAM10 structure. (C) Sequence alignment between the catalytic domains of ADAM10 (residues 207–453) and ADAM17 (residues 223–470) (identity = 40.1%).