Targeting ADAM17 Sheddase Activity in Cancer

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Abstract

A disintegrin and metalloprotease (ADAM)17 is a sheddase, capable of releasing the ectodomains of membrane proteins such as growth factors (e.g. Epidermal Growth Factor Receptor ligands), cytokines and their receptors, adhesion and signaling molecules. These activities regulate several physiological and pathological processes including inflammation, tumor growth and metastatic progression. In this review, we will summarize ADAM17 biology and focus on its role in cancer and the possible usage of ADAM17 inhibitors in cancer therapy. Recent achievements in this area include the development of small molecule metalloprotease inhibitors with enhanced specificity for ADAM17, monoclonal antibodies, and synthetic short RNA molecules for gene silencing. These approaches successfully inhibited cancer cell growth and invasiveness or sensitized them to cytotoxic drugs, ionizing radiations or targeted therapies, in preclinical studies. These findings suggest the repositioning of ADAM17 inhibitors, which have yet proven unsuccessful as anti-inflammatory agents, for the development of new anti-cancer therapies, particularly in EGFR ligand-dependent cancers. Future studies should address ADAM17 inhibitors as short-term treatments in combination with different anti-cancer therapies.

Keywords: ADAM17, cancer, small molecule inhibitor, antibodies.

1 Introduction

Metallo-endopeptidases of the Metzincin super-family [1] play key roles in physiological tissue generation and remodeling through the control of intercellular contacts and cell-matrix interactions. Any unbalance in the expression levels and/or activation status of these enzymes may participate in tissue damage in different disease conditions. These include cancer and several inflammatory or degenerative disorders, which may involve most tissues and body districts [2-7].

The Metzincin superfamily consists of five families of metalloproteases: the astacins, the reprolysins or adamalysins, the serralysins, the matrix metallo-proteinases (MMPs), also named matrixins [8, 9] and the pappalysins [10]. Pappalysins share structural similarities with the serralysins and comprise two members, the pregnancy-associated plasma protein-A1 and -A2 (PAPP-A1 and PAPP-A2).

The adamlysins or ADAMs are structurally related to the snake venomases reprolysins, and comprise two sub-families, namely the transmembrane ADAMs and the ADAMTSs, which are secreted from cells [9]. ADAMs have a prominent role as sheddases, capable of releasing the ectodomains of specific membrane proteins. Here we will focus on ADAM17, also defined as Tumor Necrosis Factor α (TNF-α) converting enzyme or TACE. Indeed, this metalloprotease was identified based on its ability to process the membrane-bound precursor protein of TNF-α to the biologically active, soluble form, and this activity was completely blocked by a metalloproteinase inhibitor [11, 12]. Subsequently, ADAM17 was shown to process Transforming Growth Factor-α (TGF-α) precursor and other Epidermal Growth Factor Receptor (EGFR) ligands, cell surface molecules such as the ErbB family of receptor tyrosine kinases EGFR [13] and HER3 [14, 15], and different adhesion molecules (*e.g.* ALCAM) [9, 16].

This review will address the ADAM17 main structural features, its sheddase activity in the context of physiological and pathological processes and highlight its role in cancer. The therapeutic potential of agents, which target ADAM17, such as natural inhibitors, small molecule metalloprotease inhibitors, monoclonal antibodies, small interfering (si)RNA and miRNA will be discussed.

2 ADAM17 structure and activation.

2.1. Transmembrane ADAMs and secreted ADAMTSs.

The two subfamilies of Adamalysins, ADAMs and ADAMTSs, display proteinase and adhesion molecule features [9, 17, 18]. The general structure and domain organization of these molecules is shown in Figure 1. As in other metalloproteases, also in Adamalysins, the pro-domain keeps the metalloproteinase domain inactive. However, a cysteine-switch mechanism is capable of uncovering the catalytic site and restoring the proteolytic activity. Between pro- and metalloproteinase domain, there is a furin recognition site (RXXR sequence), which is involved in intracellular activation of many ADAMs (ADAM9, 12, 15, 17). This process occurs in the trans-Golgi by furin-like proprotein convertases [9, 19].

The metalloproteinase domain contains a conserved HEXGHXXGXXHD sequence, also present in MMPs, which is responsible for the stabilization of the catalytic zinc ion and it is necessary for the catalytic activity [20]. Among 19 ADAMs expressed in Homo Sapiens only 12 are proteolytically active [21]. Their activity is controlled by the Tissue Inhibitor of Metalloproteinases (TIMPs) that selectively inhibit different ADAMs. For example, ADAM17 is exclusively inhibited by TIMP3, ADAM10 by TIMP1 and TIMP3, but not by TIMP2 and TIMP4, whereas ADAM8 and ADAM9 are not controlled by TIMPs [9, 22, 23]. This knowledge could be exploited for the target-oriented drug design [24- 27].

The disintegrin domain, which interacts with the integrins, mediates cell-cell and cell-matrix interactions [18, 28, 29]. However, based on the crystal structure of the snake venom homolog VAP1, it has been proposed that the disintegrin domain may be inaccessible for ligand binding, due to the peculiar folding of ADAMs [30]. Proceeding toward the C-terminus, ADAMs displays a Cysteine-rich (Cys-rich) domain important for substrate recognition [31], an EGF-like domain, a Transmembrane domain (TM) and a cytoplasmic tail containing phosphorylation sites, important for the inside-out signaling [32]. In ADAM10 and ADAM17 the EGF-like and Cys-rich domains are contained within the Membrane Proximal Domain (MPD), which is relevant for ADAM17 dimerization and substrate specificity [33]. The presence of an EGF domain in ADAM17 has been debated as the disulfide bond pattern differs from that of the typical EGF-fold. The MPD is followed by the Small Stalk Region (SSR), which also confers binding specificity to some substrates such as IL-6R, in ADAM17.

2.2. ADAM17 Catalytic Domain architecture

The structure of the catalytic domain of ADAM17, in the presence of a hydroxamic acid inhibitor, has been solved by X-ray crystallography (Figure 2) [34]. The overall structure of ADAM17 is generally similar to that of MMPs, although the conformations of the peripheral loops are different. The structures around the catalytic site are similar, and ADAM17 and MMPs bind the catalytic zinc with the same geometry. The catalytic zinc is chelated by three histidine residues in the zinc binding consensus sequence (HEXGHXXGXXHD). A conserved methionine forms the Met-turn in the active site helix, creating the active site cleft. In the globular shape, the active site is divided into two subdomains, with the active site cleft running between the two. The upper N-terminal subdomain consists of a highly twisted, 5-stranded-β-pleated sheet, with strands which lie parallel to each other. The upper subdomain also contains multiple α-helices. The lower C-terminal domain is substantially constituted by a α-helix. The catalytic zinc lies at the bottom of the groove between the two sub-domains. In other ADAM metalloproteinases, the catalytic domain is also stabilized by three disulfide bonds and by a structural Ca^{2+}, which is not present in ADAM17 and ADAM10. In all ADAMs, the active site groove is deeper on the ‘primed’ side of the zinc, corresponding to the P1', P2' and P3' residues of the substrate, than on the ‘unprimed’ side, corresponding to the P1, P2 and P3 residues. [35]. The S1' pocket of ADAM17 has an L-shape that forms a channel below Leu^{384} and Ala^{439} with the S3' pocket [34]. The crystal structures of the catalytic domain of ADAM17 in complex with many synthetic inhibitors have been reported. Most small-molecule inhibitors contain a zinc-binding ‘headgroup,’ (e.g. hydroxamate), a peptide or peptide-like backbone positioned in the ‘primed’ side of the active-site cleft, and a lipophilic substituent directed into the S1' pocket [36-38].

The role of ADAM17 domains in the sheddase activity was studied by co-transfecting cDNAs encoding for different ADAM17 deletion and chimeric constructs and known substrates in ADAM17-deficient cells from $ADAM17^{\Delta Zn/\Delta Zn}$ knockout mice. These analyses showed that different ADAM17 domains are necessary for the transformation of substrates into products, while others were not. The catalytic domain of ADAM17 is strictly necessary for substrate cleavage and could not be functionally substituted by that of ADAM10. Expression of membrane-anchored ADAM17 proteins reconstitutes shedding of different substrates, whereas secreted ADAM17 was inactive. In addition, the membrane-anchoring domain is necessary for phorbol ester-mediated activation, while the cytoplasmic domain is not required. Indeed, a chimeric molecule containing the pro-domain and the catalytic domains of ADAM17 and the downstream domains of ADAM10, but devoid of the cytoplasmic tail, mediated processing of TNF-α and TNFRII. However, the disintegrin and cysteine-rich domains of ADAM17 were necessary for interleukin 1 (IL-1)-receptor II (IL-1RII) shedding [39]. These findings also indicate that the substrate specificity of different molecular forms of

ADAM17 (isolated catalytic domain, catalytic–disintegrin–cysteine-rich domains or full-length protein) depends on its domain composition.

2.3 ADAM17 activation and regulation

ADAM17 is synthesized as an inactive precursor to avoid inappropriate substrate cleavage by the neo-synthesized protein. The processing of some substrates is dependent on the ADAM17 subcellular localization. ADAM17 is mainly found in a perinuclear compartment and on the cell surface [11, 40]. Phorbol Myristate Acetate (PMA) stimulation down-regulates ADAM17 expression on the membrane [41] and mediates its re-localization to the trans-Golgi, where it can proteolytically cleave different precursor proteins, including TNF-α precursor. The classical activation pathway of ADAM17, in the trans-Golgi network, involves the removal of the NH2-terminal pro-domain by the protein convertase furin. Pro-domain cleavage occurs at the fourth residue before the catalytic domain and makes it accessible to substrates [40]. Besides, the maturation of ADAM9, 10 and 17 also involves cleavage at an upstream site embedded in the pro-domain [42].

The second level of control is exerted at the level of ADAM17 extracellular domain, which is regulated by inter-molecular and intra-molecular interactions. A prototypical inter-molecular interaction is represented by the non-covalent complex between the catalytic region of ADAM17 and TIMP3, which inhibits its activity [43]. TIMP3 knockout animals indeed develop chronic inflammatory damage of the kidney [44, 45], the heart [46], and the liver [47, 48], due to accelerated processing of substrates (*e.g.* TNF-α, TGF-β) consequent to higher ADAM17 activity. Interestingly, TIMP3 is silenced in different human cancers via gene methylation [49] or microRNA expression [50] suggesting that it may act as a tumor-suppressor.

An alternative mechanism of ADAM17 activation, which does not involve pro-domain cleavage by convertases or removal of the endogenous inhibitor TIMP3, has been reported [51]. In murine embryonic fibroblasts, membrane ADAM17 is rapidly and reversibly activated, through a conformational change in the extracellular domain, which leads to exposure of its catalytic site. Different extracellular signals such as EGF, thrombin, lysophosphatidic acid (LPA) or TNF-α trigger this activation mechanism that is rapid and reversible, and requires the presence of ADAM17 transmembrane domain but not of the cytoplasmic tail [51].

Other binders have been described for ADAMs, such as integrins binding to the disintegrin domain [reviewed in 52]. It is worth of notice that α5β1 integrin binding to ADAM17 in mesangial cells represents a negative regulator of ADAM17 activity [53]. Intermolecular interactions are also dependent on the presence of ADAM17 in lipid rafts [54, 55] that can spatially restrict the availability of substrates. From lipid rafts, ADAM17 can also be shuttled into extracellular vesicles, which can deliver active ADAM17 to recipient cells [56].

The intra-molecular interactions involve the two highly conserved vicinal cysteine motifs of the disintegrin/MPD regions of ADAM17, whose dynamic redox changes regulate the catalytic activity of mature ADAM17. More precisely, a reducing environment diminishes whereas an oxidizing one increases ADAM17 activity [57]. In addition, Protein Disulphide Isomerases (PDI) could be responsible for maintaining ADAM17 in an inactive form. Indeed, isomerization of ADAM17 disulfide bonds induces conformational changes, which result in the rapid and reversible modulation of ADAM17 activity [58].

Post-translational modifications of ADAM17 include glycosylation and phosphorylation [reviewed in 59]. Studies based on expression of recombinant ADAM17 in different systems suggest that glycosylation may influence substrate and inhibitor binding [60, 61]. Phosphorylation of the cytoplasmic domain of ADAM17, following growth factor receptors stimulation, has also been described as a mechanism regulating enzymatic activity. It has been reported that

phosphorylation of threonine 735 [62] and serine 819 [63] is mediated by signaling through the Extracellular signal-Regulated Kinase (ERK). Also, the Phosphoinositide-Dependent Kinase 1 (PDK1), a downstream target of PI3-K, phosphorylates both residues [64]. The relevance of the cytoplasmic domain and its phosphorylation/dephosphorylation on ADAM17 enzymatic activity has been controversial [issue discussed in 51 and 59]. However, recent data indicate that down-regulation of ADAM17 membrane expression following phosphorylation by PDK1 is a key regulatory event, which decreases α-secretase activity and mediates progression in Alzheimer's disease and prion encephalopathies [65].

Several cellular signaling pathways have been linked to triggering of ADAM17 activity, including activation of G protein-coupled receptors and increased levels of free intracellular Ca^{2+} and diacylglycerol [66, 67]. Cell-surface receptors signaling via tyrosine kinase pathways are also known inducers of ADAM17 sheddase activity. For example, VEGFR-2 [68], oncogenic Kras [69] and stimuli acting via the ERK and mitogen-activated protein kinase pathways [70, 71], trigger ADAM17 activity. Src family kinases have been implicated in the activation of ADAM17 activity [72]. Indeed, transfection of an activated Src mutant resulted in increased shedding of the EGF-R ligand TGF-α through ADAM17 activation [73]. Besides, inhibition of constitutively activated Src in different tumor cell lines resulted in reduced shedding of TGF-α. Signaling through different isoenzymes of Protein Kinase C (PKC) also mediates ADAM17 activation and subsequent cleavage of EGFR ligands [74]. Recent studies propose that different stimuli can select different substrates for ADAM17 activity. Indeed activation of signaling via PKC-α triggers the processing of TGF-α, heparin-binding EGF-like growth factor (HB-EGF) and amphiregulin, while PKC-δ mediates neuregulin cleavage [75].

More recently, proteolytically inactive homologs of rhomboid proteases, termed iRHOMs, have been identified as essential controllers of ADAM17 transport and maturation [76, 77]. In particular, iRHOM1 and iRHOM2 act as chaperone proteins, which guide ADAM17 to the cell membrane, in different tissues. Indeed, iRHOM2 is essential for ADAM17 maturation in immune/inflammatory cells, whereas iRHOM1 is expressed in non-immune cells. Therefore, iRHOM2 is essential for inflammatory processes, as myeloid cells from iRHOM2-deficient mice showed defective ADAM17 activity and TNF-α release, resulting in protection from inflammatory arthritis [78]. The essential role of iRHOMs in the ADAM17-dependent EGFR signaling is evident in iRHOM1/2 double knockout mice, which show a phenotype similar to *EGFR*$^{-/-}$ and *ADAM17*$^{-/-}$ mice [79]. The different tissue distribution of iRHOMs may support the use of specific iRHOMs' blockers to inhibit ADAM17 activity selectively in different cell types. Thus iRHOM2 inhibitors may work as indirect ADAM17 inhibitors in immune/inflammatory cells, sparing ADAM17 activity in other tissues [78].

Genetic defects of the cytoplasmic domain of iRHOM1 or iRHOM2 increase ADAM17 activity and TNFR shedding, which results in reduced sensitivity to TNF-mediated death of fibrosarcoma cells [80]. Aminoterminal mutations of iRHOM2, found in patients with tylosis associated with esophageal cancer (TOC) syndrome, support enhanced TNFR shedding and TNF resistance in their keratinocytes [80].

Also, iRHOM2 is required for the LPA-stimulated shedding of HB-EGF, but not for the PMA-stimulated shedding of TGF-α, indicating that iRhom2 plays a crucial role also in controlling the substrate selectivity of ADAM17 activity [81].

Another important regulator of ADAM17 trafficking to the cell membrane, the cell sorting-protein PACS-2, was recently discovered through a genome-wide siRNA screening. Indeed, PACS-2 gene silencing resulted in reduced ADAM17 cell surface expression and EGFR ligand shedding. PACS-2 co-localizes with ADAM17 in the early endosomes and routes ADAM17 away from degradation pathways. The importance of PACS-2 in the EGFR signaling pathway was evident in PACS-2-deficient mice, which showed reduced intestinal cell proliferation [82].

Finally, recent data indicate that exposure of phosphatidylserine at the cell surface is required for ADAM17 sheddase activity through interaction with the membrane-proximal domain of the enzyme. It has been proposed that phosphatidylserine may direct the enzyme towards its targets [83].

3 Role of ADAM17 in physiological and pathological processes

ADAM17 cleaves many protein substrates, which regulate different biological processes, including inflammation and several related diseases, central nervous system functions and Alzheimer's disease, development, and tissue regeneration. These aspects will be only briefly summarized as they have been thoroughly reviewed elsewhere [59, 84, 85].

3.1 Lessons from TACE-deficient mice

ADAM17 is the main enzyme required for TNF-α processing. T lymphocytes isolated from ADAM17$^{\Delta Zn/\Delta Zn}$ mice, which bear a disruption in the ADAM17 metalloproteinase consensus sequence, show a 90% reduction in TNF-α precursor processing [11]. This finding indicates that, although ADAM10 may act as an alternative TNF-α converting enzyme, most of the TACE activity is due to ADAM17 [86, 87]. Indeed, the specificity constant for processing of a synthetic peptide, which contains the amino acid motif comprising the TNF-α precursor cleavage site, is tenfold lower for ADAM10 than for ADAM17 [88].

The analysis of the complex phenotype of ADAM17$^{\Delta Zn/\Delta Zn}$ mice revealed the existence of other ADAM17 substrates. The ADAM17$^{\Delta Zn/\Delta Zn}$ mice show perinatal/embryonic lethality and exhibit multiple defects, including open eyelids, thinned corneas, lack a conjunctival sac and defects in hair and skin [89]. These defects are similar to those found in TGF-α-deficient mice [90, 91]. Besides, ADAM17$^{\Delta Zn/\Delta Zn}$ mice display a defective maturation of the epithelia in several organs and the placental spongiotrophoblast that resembles the phenotype of the EGFR knockout mice [92-94]. Altogether, these observations point out to a defective processing of different EGFR ligands, including TGF-α, EGF, HB-EGF, and amphiregulin. Indeed, TGF-α processing is defective in ADAM17$^{\Delta Zn/\Delta Zn}$ fibroblasts [89]. The study of the ADAM17$^{\Delta Zn/\Delta Zn}$ mice allowed the identification of additional substrates, such as L-selectin [89], TNF receptors (TNF-R) I [39] and II [91], Amyloid Precursor Protein (APP) [95], IL-1RII [39], IL-6 receptor (IL-6R) [96] and Erb-B4 receptor [97]. Other reports indicated a role for ADAM17 in the processing of the Notch receptor and the osteoclast-activating cytokine TNF-related activation-induced cytokine (TRANCE) [98, 99].

Conditional KO mice displaying tissue-specific ADAM17 inactivation allowed further to dissect the role of ADAM17 activity in different cell types. ADAM17$^{flox/flox}$/Mx1-Cre or /LysM-Cr displaying inactivation of ADAM17 in cells of the myeloid lineage showed reduced lethality from LPS-mediated shock and reduced soluble TNF-α release following LPS stimulation. These data confirm the prominent role of ADAM17 in TNF-α activation and septic shock [100]. ADAM17$^{flox/flox}$/Sox-9-Cre show inactivation of ADAM17 in the non-hematopoietic compartment of the developing skeletal tissue, and have a complex phenotype including major defects in bone mass, hypercellularity of the bone marrow and extramedullary hematopoiesis. This example suggests a relevant role of ADAM17 in the development of several tissues [101]. Another example is represented by ADAM17$^{flox/flox}$/Tie2-Cre mice, which show inactivation of ADAM17 in endothelial cells resulting in reduced pathological neoangiogenesis and lung tissue damage during inflammation [102]. A recent study in ADAM17$^{ex/ex}$ mice, which display a 90% reduction of ADAM17 expression relative to wild-type mice, revealed spleen and lymph node enlargement and increased antibody responses following immunization with ovalbumin and adjuvants. ICOSL expressed on B cells is a target for ADAM17 and ADAM17ex/ex mice display higher cell surface levels of ICOSL, which may contribute to the development of excessive antibody

responses and lymphoid organ hyperplasia. These findings suggest a role of ADAM17-mediated cleavage of ICOSL in the regulation of adaptive immune responses [103].

3.2 Role of ADAM17 in inflammation and related diseases

The obvious role of ADAM17 in inflammation is linked to TNF-α maturation, whose function in rheumatoid arthritis and inflammatory bowel disease has been known for a long time [104, 105]. This knowledge has prompted the search for targeted therapies, including the use of ADAM17 inhibitors [106]. TNF-α cleavage by ADAM17 is a key event also in endotoxin shock [100] and colitis [107]. However, processing of other proteins by ADAM17 is relevant to inflammatory diseases, resulting in either pro-inflammatory or anti-inflammatory effects. Indeed, ADAM17 regulates also the bioavailability of TNF-α Receptor by shedding it from the cell surface [108]. Thus ADAM17 inhibition actually amplifies TNF-α-mediated colonic epithelial barrier disruption [109], since TNF-α could be matured by other proteases [110]. On the other hand, endothelial mitochondrial Ca^{2+} promote ADAM17-mediated shedding of TNFR1, thus regulating the severity of TNF-α-induced microvascular inflammation [111]. Indeed, endothelial deletion of ADAM17 blocked the TNFR1 shedding and augmented inflammation in mouse lung microvessels [111]. In the gut, ADAM17-mediated shedding of EGFR ligands is relevant to tissue homeostasis. Colitis, derived from defective regeneration and not linked to TNF-α maturation, is, in fact, the result of impaired shedding of EGFR ligands in conditional ADAM17$^{-/-}$ mice [112].

Another ADAM17 substrate relevant to inflammation is the IL-6R. In the case of classic IL-6 responses mediated by the cell surface receptor, IL-6R shedding by ADAM17 leads to resolution of inflammation. However, shedding can also generate an agonistic soluble (s)IL-6R, which in complex with IL-6 induces trans-signaling, through its interaction with the membrane-bound GP130 receptor, and maintains the inflammatory state [reviewed in 84]. Experiments in hypomorphic *ADAM17* and conditional *ADAM10* knockout mice showed that ADAM17 but not ADAM10 is required for IL-6R shedding during infection or endotoxemia in vivo [113]. On the other hand, ADAM10 and ADAM17 contribute to the shedding of the GP130 receptor in a soluble form, which limits IL-6 trans-signaling [114]. Recently, the details of ADAM17/IL-6R interaction have been elucidated, with the discovery of the conserved ADAM17 dynamic interaction sequence (CANDIS), which mediates IL-6R binding and is controlled by the disulfide-regulated conformation of the neighboring domain [33].

A pro-inflammatory activity of ADAM17 is linked to efferocytosis and CD36 shedding. Efferocytosis, *i.e.* apoptotic cell removal through phagocytosis, triggers immunosuppressive networks that lead to inflammation resolution. However, this process is reduced in the case of loss of the membrane-bound CD36, a scavenger receptor involved in phagocytosis. Therefore, ADAM17-mediated shedding of CD36 delays inflammation resolution by inhibiting efferocytosis. Indeed, ADAM17 deficiency leads to increased efferocytosis and supports an anti-inflammatory phenotype in a murine model of peritonitis [115], suggesting a mechanistic link between ADAM17 and persistent inflammation.

ADAM17 activity is also important in metabolic inflammation, where it acts both via maturation of TNF-α and via shedding of different substrates in major metabolic tissues, supporting the onset of Metabolic Syndrome [116]. In TIMP3$^{-/-}$ mice, the association of genetic background and dietary factors drives adipose tissue inflammation and development of obesity complications with liver inflammation and steatosis [47]. Moreover, the processing of TNF-α by ADAM17 in human skeletal muscle promotes insulin resistance, a hallmark of the metabolic syndrome [117].

Given its pivotal role in inflammation, ADAM17 is a key mediator in a number of autoimmune diseases (*e.g.* rheumatoid arthritis, Guillan-Barré syndrome, multiple sclerosis, systemic lupus erythematosus, Sjogren syndrome), as

thoroughly reviewed elsewhere [85]. Indeed, the efficacy and side effects of small molecule inhibitors of ADAM17 have been addressed in phase I and phase II trials of rheumatoid arthritis [106].

Last but not least, ADAM17 plays an important role in Alzheimer's disease due to its α-secretase activity on APP, which results in the production of a soluble, non-amyloidogenic fragment, APPsa. This neuroprotective fragment precludes deposition of aggregation-prone Ab(β) peptides [reviewed in 118]. Therefore, systemic therapeutic inhibition of ADAM17 should be afforded with caution as it could worsen amyloid pathology as a side effect.

4 ADAM17 sheddase activity and cancer

Besides its role in supporting chronic inflammation via its TNF-α-mediated effects, ADAM17 plays a prominent role in cancer development and progression thanks to its sheddase activity over other substrates involved in growth factor signaling, angiogenesis, cell adhesion and tumor escape from the immune system control. Nonetheless, ADAM17 plays a dual role in cancer progression, as it also mediates shedding of growth factor receptors involved in tumor progression or angiogenesis.

4.1 Regulation of growth factors and oncogenic signaling pathways

ADAM17-mediated shedding of ErbB ligand family members leads to receptor activation in several cancers and is associated with tumor growth [thoroughly reviewed in 13, 59, 84]. Briefly, ADAM17-mediated cleavage of EGFR ligands Amphiregulin, HB-EGF, Epigen, Epiregulin, and TGF-α regulates their bioavailability, thus playing an essential role in EGFR activation in vivo [119]. Concomitant upregulation of ADAM17 and TGF-α or HB-EGF was observed in breast [120] and ovarian cancer tissues [121], respectively. Importantly, ADAM17-dependent mobilization of TGF-α and amphiregulin in breast cancer cells may represent an autocrine loop that mediates a tumor-promoting, constitutive EGFR stimulation, independent of activating gene mutations [122, 123]. Also, shedding of TGF-α and amphiregulin, triggered by secondary bile acids, promotes tumorigenicity also in human colorectal and pancreatic ductal cells [124].

ADAM17-mediated regulation of growth factors signaling through the release of agonistic ligands may even represent a mechanism of drug resistance. In a colorectal cancer murine model, indeed, chemotherapy with 5-Florouracil (5-FU) increased ADAM17 shedding of HER ligands, and this effect significantly protected cells from apoptosis induced by either 5-FU or other drugs (*e.g.* Oxaliplatin) [125]. Moreover, in breast cancer, Herceptin triggers the ADAM17-induced release of EGF-family ligands, via a feedback loop involving PKB inhibition. As a consequence, EGFR, HER3, and HER4 receptors are activated and dimerize with HER2, promoting HER2 phosphorylation and tumor growth. The association of Herceptin and ADAM17 inhibitors abrogated EGFR-ligand activation and overcame resistance to Herceptin [126].

Another important signaling pathway targeted by ADAMs is Notch [127]. The protease required for Notch shedding is mainly ADAM10 [128, 129], but a role for ADAM17 has also been reported [99, 127, 130]. In vitro, the requirement for either ADAM10 or ADAM17 seems context-dependent. ADAM10 is absolutely required for Notch signaling induced by ligands, while ligand-independent signaling relies on ADAM17 [129]. On the other hand, knockout studies in mice do not support an active role of ADAM17 in Notch proteolysis [discussed in 131]. A recent study proposed a role for ADAM17 in supporting a baseline ('tonic') level of Notch activity of murine keratinocytes, able to maintain skin barrier functions [132]. However, this conclusion has been debated, and the reported results have been attributed to ADAM17-dependent EGFR signaling [133].

Besides the activation of oncogenic signaling, ADAM17 may also have opposite effects on cancer pro-invasive signals. Indeed, ADAM17 and ADAM10 also mediate the cleavage of c-Met, the HGF receptor involved in tumor invasiveness and progression. C-Met shedding generates a soluble fragment of the c-Met extracellular domain, which can bind to HGF and inhibit HGF-mediated pro-invasive signaling [134]. Nonetheless, in uveal melanoma, which overexpresses surface c-Met, ADAM17 and ADAM10 constitutive c-Met shedding was not sufficient to inhibit HGF-mediated invasion [135].

4.2 Regulation of neo-angiogenesis

ADAM17 also regulates angiogenesis [reviewed in 59] through the shedding of several membrane proteins with known roles in angiogenesis such as VEGFR2, ICAM-1, Tie2 and CD40 [68]. For example, ADAM17 cleavage of VEGFR2 decreases its availability on cells and generates a soluble decoy receptor, which sequesters VEGF-A and negatively regulates angiogenesis [68]. Moreover, soluble M6P/IGF2R is released by ADAM17 and controls angiogenesis by binding plasminogen and blocking its activation [136]. On the other hand, ADAM17 can support pathological neovascularization, a key event in cancer, proliferative retinopathies, and rheumatoid arthritis. Indeed, endothelial cell-specific, conditional inactivation of the ADAM17 gene reduced tumor neovascularization and growth in mice in vivo. This pro-angiogenic effect of ADAM17 is dependent on HB-EGF activation since the administration of soluble HB-EGF could rescue cell tube formation by ADAM17-deficient endothelial cells [137]. In addition, in a mouse colon carcinoma model, ADAM17 silencing was accompanied by limited angiogenesis and slower tumor growth, possibly mediated by the defective shedding of neuregulin-1 [138]. In glioma cells, overexpression of ADAM17 promotes the malignant phenotype as it increases VEGF expression level, which mediates tube formation by endothelial cells in vitro, and, more importantly, sustained U87 glioma cell line tumorigenesis in a xenograft model. SiRNA-mediated silencing of ADAM17 reduced shedding of TGF-α, decreased EGFR and Akt phosphorylation and reduced in vivo glioma growth [139]. Recent data indicate that ADAM17 promotes sprouting angiogenesis by down-regulation of the natural inhibitor of angiogenesis thrombospondin-1 (TSP1). Indeed, ADAM17 inhibition is followed by a decrease in blood vessel density and sprouting, in vivo [140]. Moreover, genetic variants of ADAM17 are hypomorphic in down-modulating TGF-β signaling through differential shedding and consequent inactivation of TGFβRI, and this lowers the angiogenesis potential and the circulating endothelial progenitor cell numbers in mice [141].

Finally, ADAM17 activity promotes the migratory and sprouting potential of lymphatic endothelial cells through the shedding of EGFR ligands and regulation of integrin surface expression. Therefore, ADAM17 silencing has an anti-lymphangiogenic effect [142]. Altogether these findings support a pro-angiogenic activity of ADAM17 in pathological conditions, such as cancer.

4.3 Intercellular adhesion molecule shedding

Regulation of cell adhesion is relevant both to primary tumor growth and to metastatic dissemination. In this context, ADAM17 is the sheddase of many adhesion molecules (*e.g.* L1-CAM, CD44, CD62L, ICAM-1, and VCAM-1) [reviewed in 143] and may therefore be instrumental in releasing the constraints that limit cell motility. Moreover, soluble adhesion molecules generated by ectodomain shedding contribute to cancer progression, since they may act as negative competitors that impair cell adhesion [reviewed in 144]. In addition, ADAM17-mediated intra-membrane proteolysis of adhesion molecules such as EpCAM, release its intracellular domain, whose nuclear translocation acts as a mitogenic signal transducer [145].

An example of the role ADAM17 activity in tumor cell adhesion is the shedding of Activated Leukocyte Cell Adhesion Molecule (ALCAM). Indeed, recombinant ADAM17 mediates proteolysis of purified ALCAM [146] and small molecule inhibitors of ADAM17 or ADAM17 silencing block ALCAM cleavage in cancer cells in vitro [16]. ALCAM homophilic and heterophilic (ALCAM-CD6) interactions mediate intercellular adhesion processes, and the regulation of ALCAM dynamics has a central role in tumor cell motility and invasive properties. Both shedding and internalization of ALCAM are involved in the release of cell-to-cell contacts and permit cancer cell migration and invasion. Indeed, ALCAM is expressed at the cell surface of epithelial ovarian cancer (EOC) cells, and can be internalized, upon soluble ligand binding [147]. This process may be relevant for in vivo aggressiveness of cancer cells, as cytoplasmic staining for ALCAM in tumor tissue correlated with a poorer outcome in EOC, in comparison with membrane localization [148]. Similarly, elevated ALCAM shedding correlates with a poorer outcome in colorectal cancers [149]. ADAM17-mediated shedding of ALCAM leads to the release of the soluble ectodomain of ALCAM (sALCAM). In turn, sALCAM is capable of homophilic interaction with membrane ALCAM, and may, therefore, act as a decoy and induce ALCAM internalization, thus contributing to the negative regulation of cell adhesion. Indeed, a recombinant anti-ALCAM antibody or a soluble ALCAM-Fc chimera, which block ALCAM-mediated adhesion, enhanced motility and invasiveness of EOC cells in vitro [16, 150]. EGF stimulation activates ADAM17, which increases ALCAM shedding and in vitro invasiveness. These effects are blocked by ADAM inhibitors or by ADAM17 gene silencing, further suggesting that ADAM17 may represent a therapeutic target, particularly for EGF-dependent tumors [13]. A recent report showed that TGF-β is also a stimulus for ADAM17-mediated ALCAM shedding, which is relevant for metastatic dissemination in a prostate cancer model [151].

4.4 Role of ADAM17 in cancer-related immune regulation

Besides cleavage of growth factors receptors and ligands and adhesion molecules, ADAM17 plays a role in cancer progression through the shedding from the cell surface of some natural killer cells-activating receptors or of their ligands on the cancer cells. This activity represents a potential mechanism of tumor escape as shed ligands may interfere with tumor cell recognition by NK cells and cytotoxic T cells expressing ligand specific receptors. The MHC class I-related chain molecules A and B (MICA and MICB) are ligands of the NKG2D receptor and their interaction activates the cytolytic functions of NK cells and subsets of cytotoxic T cells. The surface expression of MICA and MICB is reduced by proteolytic cleavage and the release of the soluble ectodomain can further down-modulate NKG2D expression [reviewed in 152], thus contributing to escape from NK cell surveillance. Indeed, ADAM10 and 17 mediate the release of soluble MICA/B, and blockade of their enzymatic activity stabilizes MICA cell surface expression on cancer cells. These events enhance NKG2D signaling and NK cell recognition [153, 154]. However, processing of MICA and MICB is differentially regulated by ADAM17 and ADAM10 in different tumors [154, 155]. Also, a recent study reported that ADAM17 and ADAM10 mediate the proteolytic removal from the tumor cell surface of B7-H6, a ligand for the activating receptor NKp30 that triggers NK-cell-mediated killing. Pharmacological or genetic inhibition of the shedding process restores the B7-H6 cell surface expression and the NKp30-mediated NK cell activation [156].

Besides its activity on ligands of NK receptors, ADAM17 can regulate NK activity through the cleavage of the FcγRIIIA (CD16), a receptor that binds to IgG immune complexes and is crucial for NK cell-mediated killing of antibody-coated target cells. This process is defined antibody-dependent cell mediated cytotoxicity (ADCC) and is involved in the therapeutic activity of several tumor-targeting therapeutic monoclonal antibodies [157, 158]. Therefore, it has been proposed that targeting ADAM17 may contribute to enhance the efficacy of therapeutic antibodies. Indeed, recent data showed that treatment with an ADAM17 inhibitor blocked CD16 shedding thus increasing NK cell

activation against CD33+ leukemia cells mediated by a novel bispecific killer cell engager linking CD16 to CD33 [159]. Altogether these data support a role of ADAM17 expressed by cancer cells in the impairment of tumor recognition by the immune system through multiple mechanisms.

However, NK cells from a patient with a very rare genetic deficiency of ADAM17 showed high levels of CD16 expression and activation but no substantial increase in ADCC activity [160]. In addition, leukocytes from this patient had impaired cytokine secretion following LPS stimulation, in accordance to the severe bacteremia shown by the patient. This observation suggests caution in the clinical use of ADAM17 inhibitors for boosting ADCC activity.

5 ADAM17 targeting strategies

The vast majority of small molecules synthesized so far as selective ADAM17 inhibitors were tested in chronic inflammatory diseases, including rheumatoid arthritis (RA), psoriasis and Crohn's disease [161]. The success of anti-TNF-α biological agents, such as etanercept and infliximab, in alleviating the clinical symptoms of RA strongly boosted the research of orally bioavailable small synthetic molecules able to reduce the circulating level of TNF-α by inhibiting ADAM17. In the last decade, many ADAM17 selective inhibitors have been reported, but no one has shown efficacy in Phase II clinical trials in RA. Besides new small molecule inhibitors, monoclonal antibodies, modified TIMPs, and small non-coding RNA targeting ADAM17 have been recently developed and tested in experimental cancer models in vitro and in vivo.

5.1 Small molecule inhibitors

The structures of some nanomolar ADAM17 selective inhibitors representative of the principal chemical classes developed so far are depicted in Table 1. Most of these compounds are hydroxamate-based zinc-binders with an amide or sulfonamide group as hydrogen bond acceptor and a hydrophobic group able to interact with the S1' pocket of ADAM17. Based on the crystal structure of the catalytic domain of ADAM17 [34], many efforts have been directed to find leads able to inhibit ADAM17 and spare MMPs by exploiting the differences between the S1' pockets of these two families of enzymes. In fact, ADAM17 has an "L-shaped" pocket with a polar entrance between S1' and S3'. The peculiar conformation of the S1' pocket has been exploited to design selective inhibitors by the introduction of bent substituents in P1'. ADAM17 selectivity over MMPs has been considered important to avoid the side effects found in clinical studies of MMP small molecule inhibitors with a broad specificity [187].

The first ADAM17 inhibitors were succinate-based hydroxamates, such as Ro 32-7315 and TAPI-2 (Table 1), with a moderate activity against some MMPs. Bristol-Myers Squibb developed several amide-based hydroxamates (compounds 3-8, Table 1) all having a 4-(2-methylquinolin-4-ylmethoxy)phenyl group as P1' substituent, which was found to impart selectivity for ADAM17 and improve activity. Among these, BMS-561392 (DPC-333) was a potent (IC50=0.2 nM) ADAM17 inhibitor with a γ-lactam core, which entered clinical trials for RA. Despite its excellent potency and selectivity toward ADAM17 over MMPs in vitro, BMS-561392 was withdrawn from clinical trials because of its hepatotoxicity [106].

Scientists at Wyeth reported some sulfonamide (compounds 11, 12 and 13) and sulfone hydroxamates (compounds 18 and 19, Table 1) bearing a 4-(but-2-ynyloxy)phenyl group as P1' substituent, given its good fit with the "L-shaped" S1' pocket of ADAM17. Among these, TMI-2 (WAY-022, Table 1) had an improved selectivity for ADAM17 and was also effective in the collagen-induced arthritis model [179, 180].

More recently different cyclopropane-based hydroxamic acid inhibitors were reported by Schering-Plough [188]. After a lead optimization process, a cyclopropyl derivative (compound 25, Table 1) endowed with nanomolar affinity (Ki=4.0 nM) and good pharmacokinetic profile after oral administration in mice was discovered.

Several studies have tried to replace the hydroxamic acid moiety with other Zinc-Binding Groups (ZBG) to overcome the instability and toxicity issues linked to the use of hydroxamates. Duan *et al*. reported a potent (IC50=2.0 nM) ADAM17 inhibitor (compound 20, Table 1), obtained by combining a 4-(2-methylquinolin-4-ylmethoxy)phenyl group as the P1' substituent with a pyrimidine-2,4,6-trione as ZBG [181]. In 2012 Minond *et al*. published the first non-zinc-binding ADAM17 inhibitor (compound 23, Table 1), a piperazine-2,3-dione derivative devoid of any ZBG [60]. This compound was discovered through high-throughput screening assays using glycosylated and non-glycosylated substrates. Compound 23 was a low micromolar (IC50=4.2 μM) inhibitor of ADAM17 selective over ADAM10, MMP14, and MMP8. The following SAR study obtained six new lead compounds, which were fully characterized using cell-based assays [189]. The secondary binding site (exosite) inhibitors devoid of ZBG could represent a valid option to achieve a substrate-selective inhibition of ADAM17.

The first studies in cancer models were performed with early ADAMs/MMPs inhibitors such as TAPI-1 and TAPI-2 (Table 1). These compounds inhibited the shedding of EGFR ligands, and the PI3K-AKT pathway downstream the EGFR, in breast cancer cells [122, 190].

Merchant *et al*. showed that the combined use of a monoclonal antibody targeting the EGFR, an EGFR tyrosine kinase inhibitor, and an ADAM17 inhibitor (TMI-2 or WAY-022, Table 1) mediated a cooperative growth inhibition and apoptosis of colon cancer cells [191]. Besides, TMI-2 has been used to validate ADAM17 as a therapeutic target for triple-negative (TN) breast cancer. McGowan *et al*. showed that ADAM17 is expressed at high levels in TN breast cancers, and TMI-2 inhibited the release of TGF-α and proliferation of these cancer cells [192]. Therefore, inhibition of ADAM17, especially in combination with chemotherapy or EGFR/HER inhibitors, could represent a new therapeutic option in TN breast cancer. Also, TMI-1 (compound 11, Table 1) selectively inhibited tumor cells and cancer stem cell survival and proliferation at submicromolar doses while non-malignant cells were resistant. Interestingly, treatment of transgenic MMTV-ERBB2/neu mice prevented spontaneous mammary tumor development and induced tumor cell apoptosis. Moreover, TMI-1 had a strong synergistic effect in association with docetaxel, doxorubicin, and lapatinib [193].

A recent report showed that irradiation of lung cancer cells increased furin-mediated activation of ADAM17, which promoted shedding of multiple survival factor mediators of radio-resistance. Indeed, TMI-005 (compound 12, Table 1), a dual ADAM17 and MMP-13 inhibitor developed as an anti-inflammatory drug [173], sensitized non-small cell lung cancer cells to ionizing radiation, in vitro and in xenograft models [194]. Indeed, this study opens new perspectives for combinational therapies exploiting ADAM17 inhibitors and radiotherapy.

One of the most widely investigated inhibitors in cancer models is INCB3619 (compound 9, Table 1) an orally active potent dual inhibitor of ADAM17 (IC50=14 nM) and ADAM10 (IC50=22 nM) with low activity on other ADAMs and MMPs. This compound blocked the release of TGF-α, HB-EGF, amphiregulin, and heregulin at nanomolar concentrations in cell-based assays. Administration of INCB3619 to tumor-bearing mice inhibited tumor growth without musculoskeletal side effects. Moreover, it synergized with cisplatin in head and neck cancers, and with paclitaxel in breast cancer [15] and non-small cell lung cancer xenograft models [14]. An ADAM17 selective inhibitor (compound 17, Table 1) proved to be effective, at nanomolar concentrations, in reducing ADAM17-mediated ALCAM shedding in human ovarian cancer cell lines [178]. ALCAM shedding is important for EOC cell migratory properties and invasiveness, and can be blocked by using ADAM17 inhibitors [150].

Although the clinical studies of ADAM17 small molecule inhibitors failed to show efficacy in inflammatory diseases, preclinical studies unveiled a potential role of ADAM17 in different type of cancers, particularly in combination with chemotherapy or targeted therapies.

5.2 Monoclonal antibodies and TIMP3

The high degree of homology between the active sites of metalloproteases has limited the development of highly specific small molecule inhibitors for ADAM17. The generation of antibodies, which specifically recognize or inhibit ADAM17, is an attractive possibility to target ADAM17-expressing cells. Anti-ADAM17 antibodies may be used as specific inhibitors of enzymatic activity or for targeting toxic compounds or cytotoxic T cells against cancer cells.

To produce selective inhibitors of ADAM17 activity, a human monoclonal antibody was selected using a two-step approach from single-chain fragment variable (scFV) antibody phage-display libraries [195]. In a first step, an ADAM17 molecule bound to a small molecule inhibitor blocking the catalytic site was used for screening. This procedure allowed the selection of a scFV (D1), which recognizes a non-catalytic portion of ADAM17 (within the Disintegrin and Cysteine-rich domains) through its VH region. The VH sequence of this antibody was then used to construct a second VH-biased library with different new VLs. In the second step, this library was screened using recombinant ADAM17 with an active, unblocked, catalytic site. The scFv antibody isolated by this second screening D1(A12) is capable of recognizing the catalytic domain via its VL portion and cross-binding the ADAM17 non-catalytic domain trough the VH. The D1(A12) scFv is a specific and potent inhibitor of ADAM17 activity, as detected by a TNF-α-conversion assay [195]. The scFv was then used to construct a fully human IgG1 antibody, which displayed a five-fold higher inhibitory activity on ADAM17, relative to TIMP-3. Indeed, D1(A12) blocked the cleavage of several ADAM17 substrates from an ovarian cancer cell line, with an IC50 for TNF-α in the nM range [196]. The pharmacokinetics and anti-tumor activity of the D1(A12) IgG1 were studied in an ovarian cancer xenograft model. A single dose of 10 mg/kg produced plasma and ascites levels over 100 nM for at least one week. D1(A12) IgG1 administered at weekly intervals inhibited ovarian cancer growth and the release of human ADAM17 substrates, including the EGFR ligands amphiregulin and TGF-α. These data indicated that D1(A12) IgG1 has anti-tumor activity and may be best suited for the therapy of EGFR expressing tumors [196]. Interestingly, D1(A12) showed anti-tumor activity in vitro in triple-negative breast cancer cells, which represent an aggressive type of cancer lacking estrogen and progesterone receptors and HER2 amplification [197]. MEDI3622 is another anti-ADAM17 inhibitory antibody, which inhibited tumor growth in several EGFR-dependent tumor models. It performed better than EGFR/HER pathway inhibitors in esophageal and colorectal cancer models, suggestive of EGFR pathway-independent activity. In addition, MEDI3622 showed additive effects with the anti-EGFR mAb Cetuximab, leading to tumor eradication [198].

In human cancer xenografts in immune-deficient mice, D1(A12) inhibited ADAM17 mediated shedding of surface molecules from human tumor cells but did not inhibit TNF-α release from mouse inflammatory infiltrates. To overcome this limitation and to further investigate the use of anti-ADAM17 antibodies in syngeneic preclinical models, a recombinant antibody reactive with both mouse and human ADAM17, termed A9, was selected. The A9 scFv was then formatted in a mouse IgG2 backbone and optimized by in vitro affinity selection [199]. The A9 IgG antibody strongly inhibited cell surface ADAM17 activity in human and mouse cancer cells and is, therefore, suitable for in vivo studies in murine pre-clinical cancer models.

Besides the possible usage as inhibitors of enzymatic activity, anti-ADAM17 antibodies can be used for tumor targeting of cancer cells that overexpress ADAM17, including prostate, pancreatic, breast, ovarian and non-small lung cancer cells. Upon binding with cell surface ADAM17, the A300E antibody is rapidly internalized in ADAM17-expressing

tumor cells and for this reason, it can be utilized for the delivery of toxic compounds. Indeed, doxorubicin- or Pseudomonas exotoxin A-coupled A300E selectively eliminates ADAM17-expressing MDA-MB-231 breast cancer cells [200]. Thus, anti-ADAM17 antibodies may be utilized for selective drug delivery to cancer cells.

Another strategy is based on the targeting of effector lymphocytes against cell-surface antigens of tumor cells. This goal can be achieved by the use of bispecific mAbs, which bind with one arm to a surface receptor that activates the lymphocyte and with the second arm to a tumor-associated surface antigen [201]. In particular, the bispecific mAb A300E-BiTE (bispecific T-cell engager antibody) is a fusion protein formed by an anti-ADAM17 and an anti-CD3 scFv [202]. A300E-BiTE mediated ADAM17-specific killing of prostate tumor cells by primary human T-cells and may represent a novel strategy for the treatment of ADAM17-expressing tumors.

TIMP3 is a matrix-bound inhibitor of metalloproteases, including different MMPs, ADAM17 and ADAM-TS [reviewed in 203]. Its expression is down-regulated in several cancer cells through gene methylation [49] or miRNA expression [50], leading to the hypothesis that it may act as a tumor suppressor gene. For example, recent findings indicate that miR21, which targets TIMP3 mRNA, is overexpressed in aggressive cutaneous melanoma. Also, transduction of miR21 down-regulated TIMP3 expression and increased invasiveness of cancer cells [204]. Other reports indicated that transduction of TIMP3 gene in glioma [205] or melanoma cells reduced tumor invasiveness or growth and induced apoptosis [206]. TIMP3 has also been engineered with the aim to generate mutants with diverse specificities towards different ADAM/MMPs family members to generate potential therapeutics [203]. However, TIMP3 has not yet been tested in clinical trials in cancer patients.

5.3 Gene silencing approaches

Synthetic non-coding RNAs such as small-interfering RNA (siRNAs), short-hairpin RNA (shRNA) and microRNA (miR) can mediate a sequence-specific gene silencing and suppress encoded protein expression by acting at the transcriptional or post-transcriptional level [reviewed in 207]. This approach is widely used as a research tool for in vitro studies of gene and protein function.

For example, ADAM17 silencing using siRNA transfection demonstrated the involvement of ADAM17 in hypoxia-mediated cisplatin resistance through the activation of the EGFR signaling pathways, in hepatocellular carcinoma cells [208]. Moreover, ADAM17 silencing inhibited ALCAM shedding, which is required for cell motility, from ovarian cancer cells [16]. In prostate carcinoma cells, silencing of ADAM17 exerted antiproliferative effects through the inhibition of EGFR/PI3K/AKT signaling [209]. Also, siRNA–mediated silencing of ADAM17 inhibited migration and proliferation of the MCF7 breast cancer cell line in vitro and reduced their growth in nude mice, by acting on the EGFR pathway [210].

A recent report showed that targeting of ADAM17 through siRNA or the small molecule inhibitor TAPI-2 reverted the sphere-forming phenotype of colorectal cancer stem cells and enhanced their sensitivity to 5-fluorouracil, through inhibition of Notch-1 signaling [211].

Other reports indicated that different miRNAs regulate the expression of ADAM17. Indeed, in vitro transfection of synthetic mimics of miR-145 [212, 213], -152 [214], -221/222 [215] or -122 [216] targeted ADAM17 mRNA and inhibited ADAM17 expression. This effect resulted in the inhibition of the proliferation, migration, and invasiveness of different cancer cells, including hepatocellular carcinoma [213, 216], nasopharyngeal carcinoma [217], glioma [212], breast [215] or lung [214] cancer cells. In general, the expression of these miRNA was down-regulated in cancer cells, and the anti-tumor effects evidenced by miRNA transfection suggested that they may behave as onco-suppressors, in specific tumors.

A recent report used lentiviral vectors for the transduction of shRNA targeting ADAM17, in lung cancer cells [218]. This approach reduced cell proliferation, migration, and invasion in vitro and also suppressed tumor growth in vivo. Finally, knock-down of ADAM17 through shRNA inhibited the pro-metastatic and tumorigenic effects of the oncogene-induced senescence secretome, in breast cancer cells. These data outline the role of ADAM17 in the release of a pro-metastatic secretome [219]. Altogether these data indicate that synthetic non-coding RNAs targeting ADAM17 should be further studied as potential anti-cancer agents. In general, gene-silencing technologies based on synthetic RNAs represent an emerging therapeutic approach. Although the development or the refining of novel RNA delivery systems is still in process [220], Phase I clinical studies using different liposomal-based small RNA delivery systems are ongoing for different targets and disease conditions. It is likely that the evolution of these new systems will provide novel tools that may lead to in vivo therapeutic applications for ADAM17 gene suppression in cancer cells.

Concluding remarks

ADAM17 plays an important pathogenic role in the inflammatory response associated with different autoimmune diseases and in cancer, through the activation of TNF-α and the EFGFR-related ligands. Although preclinical studies of ADAM17 small molecule inhibitors showed promising findings in animal models of inflammatory diseases, clinical studies in RA showed no significant benefit and revealed hepatic toxicity. The possible reasons for this toxic effect are still unknown, but they may relate to possible effects on other targets, as hypomorphic ADAM17$^{ex/ex}$ mice show no signs of liver damage. It is possible that these problems may be solved by the design of novel small molecule inhibitors with a different molecular structure and enhanced target specificity. Nonetheless, the multiplicity of ADAM17 substrates and their different physiological roles may still represent a substantial problem, particularly in patients requiring long-term treatments. Perhaps, in this respect, targeting of ADAM17 exosite, rather than the catalytic site, may allow creating compounds with restricted substrate specificity.

Although broad-spectrum MMPs inhibitors failed in clinical studies of cancer, ADAM17 selective inhibitors were not clinically tested in cancer. In this respect, several preclinical studies indicated that ADAM17 inhibitors are active in suppressing epithelial cancer cell growth and/or invasiveness or may sensitize them to the activity of cytotoxic drugs, ionizing radiations, or targeted therapies. These data strongly support the repositioning of ADAM17 small-molecule inhibitors as sensitizing agents for different anti-cancer therapies. In particular, given the important role of ADAM17 in the activation of EGFR ligands, ADAM17 inhibitors should be tested in EGFR-dependent cancers. It is conceivable that short-term treatment with the ADAM17 inhibitors may be utilized in cancer in combination with other treatments, thus limiting the development of toxicity related to chronic use.

Interestingly, ADAM17 inhibitors may also enhance the activity of effector cells of the innate immune system, such as NK cells, by limiting the release of soluble ligands for NK receptors from tumor cells or by increasing the expression of activation receptors (*e.g.* CD16) on NK cells. Therefore, ADAM17 inhibitors may potentiate the effect of therapeutic monoclonal antibodies that target a tumor antigen and elicit ADCC activity by NK cells by engaging the CD16 receptors. This is the rationale for an ongoing clinical trial testing the combination of the ADAM17 inhibitor INCB7839 with rituximab, after autologous hematopoietic cell transplantation, in diffuse large B-cell lymphoma patients (NCT02141451).

Besides small molecule inhibitors, anti-ADAM17 monoclonal antibodies represent a new class of highly specific inhibitors of enzymatic activity. In addition, these antibodies could also be utilized for the targeting of toxic compounds or cytotoxic T cells against cancer cells overexpressing ADAM17, through the generation of immunotoxins or bispecific monoclonal antibodies, which showed activity in pre-clinical studies.

Recent findings on the role of iRhom 1 and 2 as tissue-specific chaperons, regulating ADAM17 expression on the cell membrane, may shift the attention to these molecules as therapeutic targets for the design of tissue-specific ADAM17 inhibition. For example, targeting of iRhom 2 may allow suppression of ADAM17 expression in inflammatory cells such as monocytes/macrophages and granulocytes, while sparing expression on epithelial cells.

Finally, a recent strategy for inhibiting ADAM17 expression in cancer cells is represented by gene silencing approaches. These strategies showed interesting results in pre-clinical models but still require the refining of new tools, suitable for in vivo RNA delivery to cancer cells in clinical studies.

Conflict of Interest

The authors have no conflict of interest to declare.

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Figure legends

Figure 1. Structure and domain organization of ADAM and ADAMTS family members. ADAMs display common domains: Propeptide (Pro), Metalloproteinase (Metallo), Disintegrin (Dis) (an RGD domain is present in ADAM15), Cysteine-rich (Cys-rich), EGF-like (EGF), Transmembrane (TM) and Cytoplasmatic domains. In the structurally related ADAM10 and ADAM17 the Membrane Proximal Domain (MPD) comprises Cys-rich and EGF-like sequences, and a Small Stalk Region (SSR) is also present. ADAMTS differ from ADAMs as they lack EGF, TM, and cytoplasmic domains and display Thrombospondin-1 motifs (TSP1) and a Spacer domain (SP). Some members of these families also contain a recognition motif for furin-like enzymes (Fu).

Figure 2. Crystal structure of the catalytic domain of ADAM17 complexed with a peptidomimetic hydroxamate (PDB code: 1BKC. In evidence the catalytic sequence H^{405}-E^{406}-H^{409}-H^{415} and the catalytic Zn^{2+} ion in a pentacoordinate complex with the hydroxamate moiety of the inhibitor).

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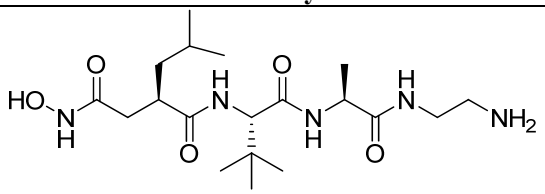
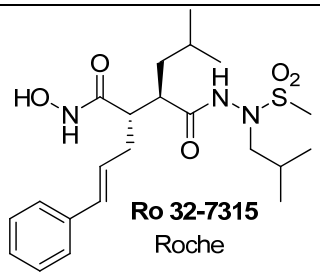
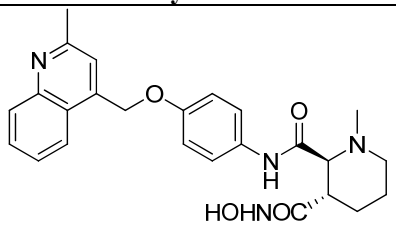
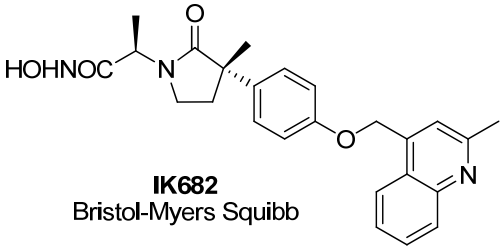
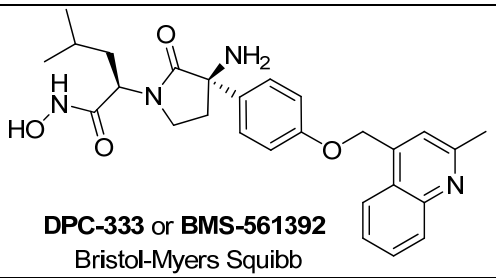
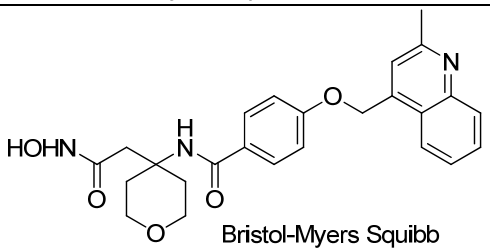
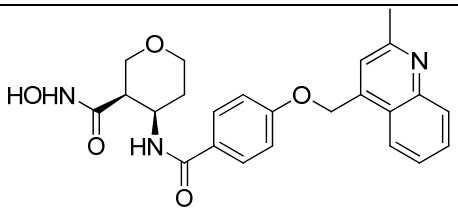
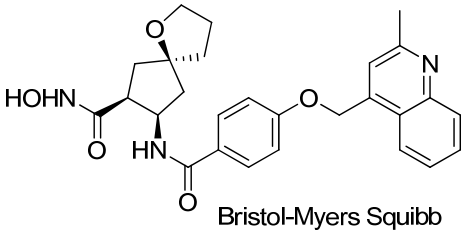
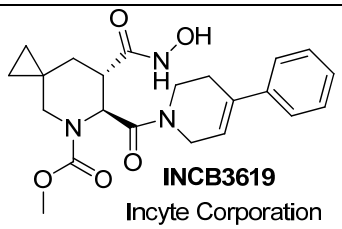
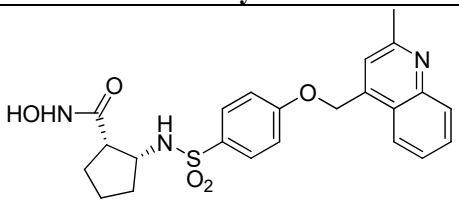
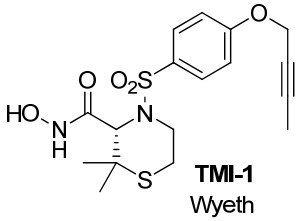
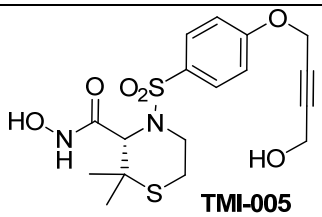
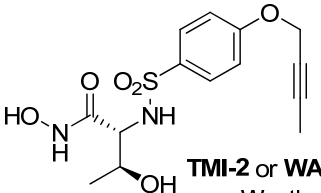
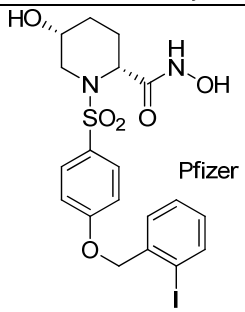
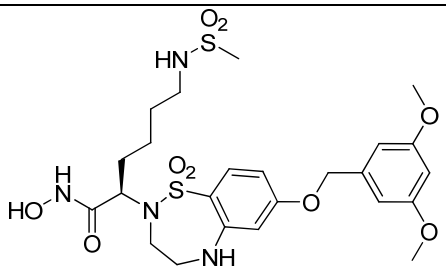
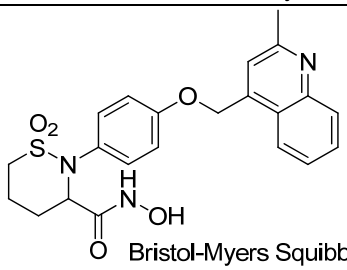
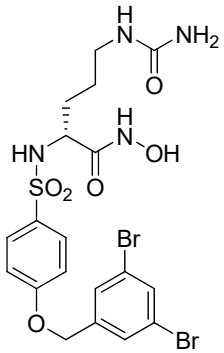
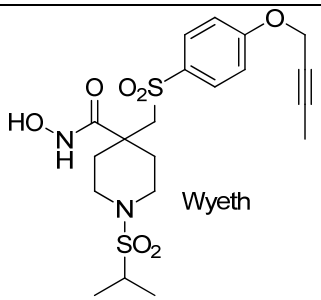
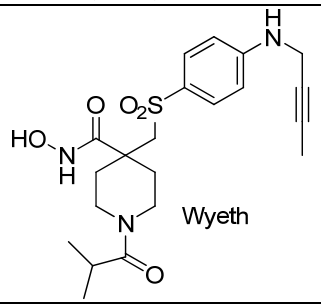
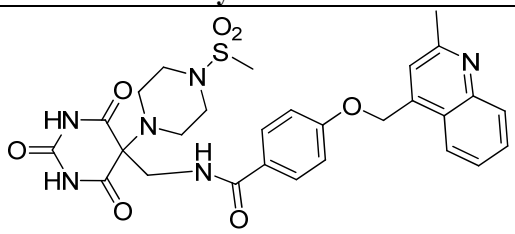
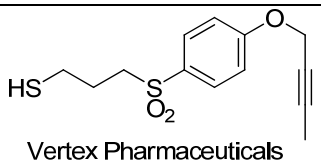
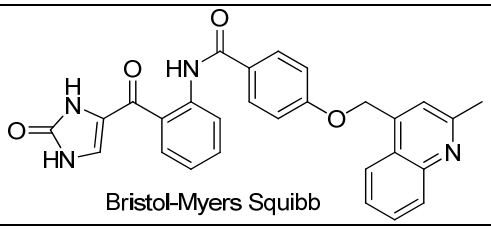
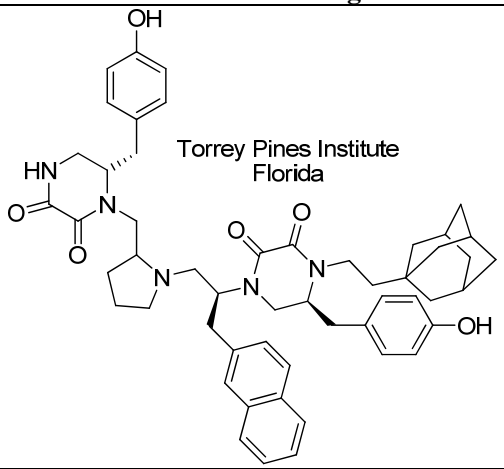
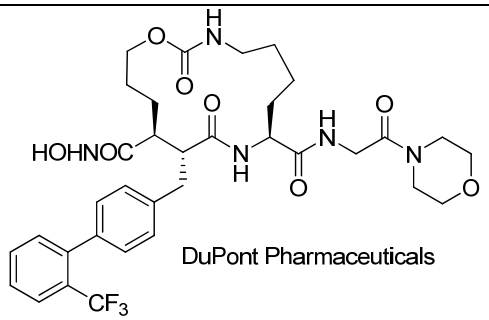
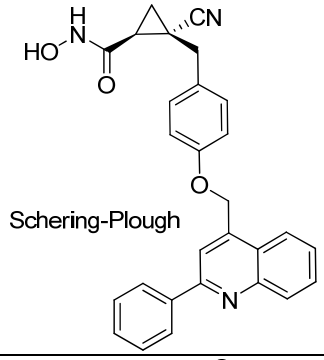
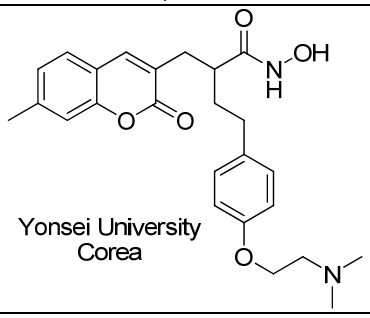
Table 1. Small molecule ADAM17 selective inhibitors.

ID	Inhibitor	In vitro potency on ADAM17: IC_{50} (nM)	References
	Succinate-based hydroxamates		
1	 **TAPI-2** Immunex Corporation	120 (K_i)	[162]
2	 **Ro 32-7315** Roche	5.2	[163]
	Amide hydroxamates		
3	 **IM491** Bristol-Myers Squibb	6.2*	[164]
4	 **IK682** Bristol-Myers Squibb	1.0*	[165]

5	 **DPC-333** or **BMS-561392** Bristol-Myers Squibb	0.20	[166]
6	 Bristol-Myers Squibb	0.35* (K_i)	[167]
7	 Bristol-Myers Squibb	0.15*(K_i)	[168]
8	 Bristol-Myers Squibb	1.0*	[169]
9	 **INCB3619** Incyte Corporation	14	[170]
	Sulfonamide hydroxamates		
10	 **WAY-281418** Wyeth	0.6	[171]
11	 **TMI-1** Wyeth	8.4	[172]

12	 **TMI-005** Wyeth	20	[173]
13	 **TMI-2** or **WAY-022** Wyeth	2.0	[174]
14	 Pfizer	7.0	[175]
15	 Bristol-Myers Squibb	5.0* (K_i)	[176]
16	 Bristol-Myers Squibb	3.7	[177]
17	 University of Pisa, Italy	1.9	[178]
	Sulfone hydroxamates		

18		1.5	[179]
19		2.3	[180]
	Non-hydroxamates		
20		2.0*	[181]
21		10 (K_i)	[182]
22		9.0*	[183]
	Non-zinc-binding		
23		4200	[60]
	Miscellaneous		

24		2.8* (K_i)	[184]
25		4.0 (k_i)	[185]
26		0.3	[186]

*porcine ADAM17

Figures

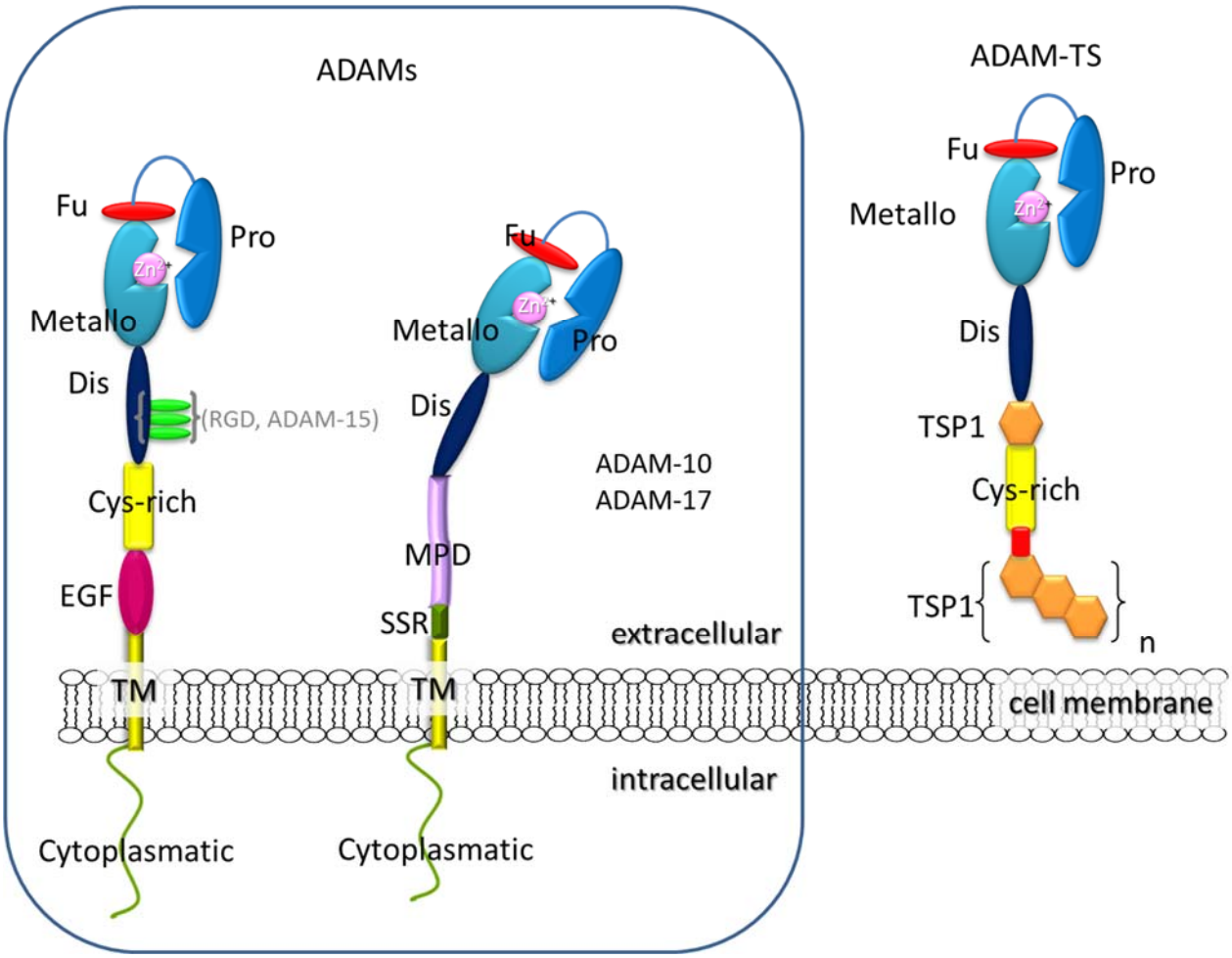


Figure 1

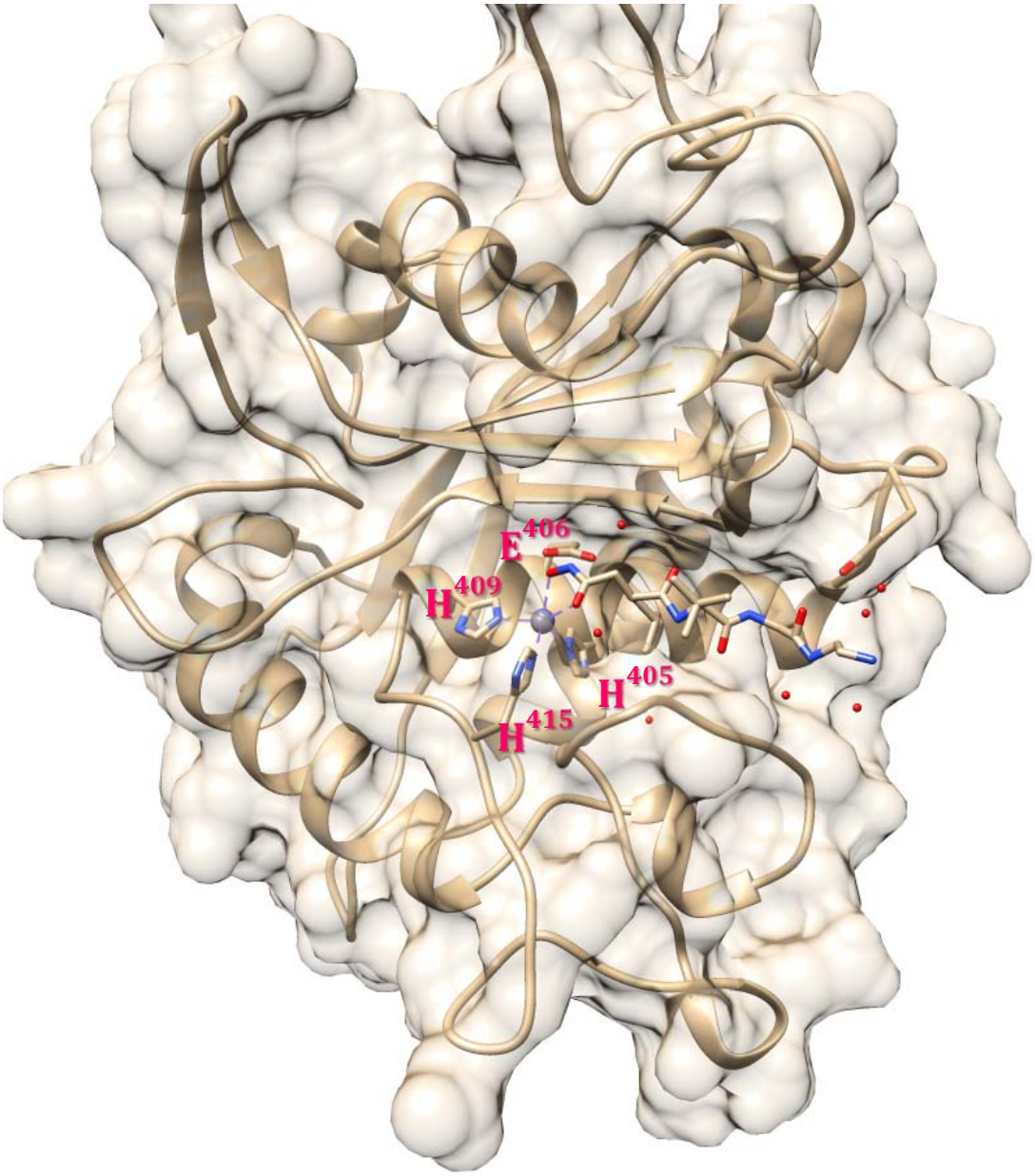


Figure 2