TACE and other ADAM proteases as targets for drug discovery

Marcia L. Moss, Judith M. White, Millard H. Lambert and Robert C. Andrews

Tumor necrosis factor (TNF)-converting enzyme (TACE) and other ADAM proteases (those that contain a disintegrin and a metalloprotease domain) have emerged as potential therapeutic targets in the areas of arthritis, cancer, diabetes and HIV cachexia. TACE is the first ADAM protease to process the known physiological substrate and inflammatory cytokine, membrane-bound precursor-TNF-α, to its mature soluble form. Subsequently, TACE was shown to be required for several different processing events such as tumor growth factor-α (TGF-α) precursor and amyloid precursor protein (APP) cleavage. With the recent discoveries of the proteolytic specificities of other ADAM family members, the information surrounding these metalloproteases is expanding at an exponential rate. This review focuses on TACE and other family members with known proteolytic function as well as the inhibitors of this class of enzyme.

***Marcia L. Moss**
Cognosci
2 Davis Drive
PO Box 12076
Research Triangle Park
NC 27709, USA
*tel: +1 919 362 1339
e-mail: Moss0610@yahoo.com
Judith M. White
Dept of Cell Biology
UVa Health System School of Medicine
Charlottesville, VA, USA
Millard H. Lambert and Robert C. Andrews
Glaxo SmithKline
Research Triangle Park
NC, USA

▼ A large group of type I integral membrane proteins are called ADAMs because they contain a disintegrin and a metalloprotease domain[1]. Several ADAMs will be discussed in detail in this review because they have emerged as therapeutic targets for a variety of diseases including Alzheimer's disease, Crohn's disease and arthritis. Currently, there are 29 distinct ADAM family members represented in public databases. ADAM-TS proteins comprise a related family of secreted proteins with an ADAM-like metalloprotease domain followed by a domain that includes thrombospondin (TS) repeats[2]. ADAMs have been found in a variety of organisms including *Caenorhabditis elegans*, *Drosophila*, *Xenopus laevis* and humans.

Among the 29 ADAMs, 17 have the consensus sequence, HEXGHXXGXXHD (in single letter amino acid code and where X represents any amino acid), which is predicted to be the catalytic site of an active Zn-dependent metalloprotease. Four of the candidate ADAM metalloproteases have been tested for protease activity, and because all displayed activity, at least *in vitro*, it is probable that all ADAMs with the consensus catalytic sequence are active metalloproteases. With one possible exception[3], possession of protease activity appears to be conserved among members of a given subfamily. Some ADAMs are found predominantly at the cell surface whereas others appear to reside primarily in an intracellular compartment (presumably the Golgi apparatus). ADAMs with metalloprotease activity are thus unique among membrane, and especially cell surface, proteins in possessing two seemingly antithetical domains: an adhesive domain (disintegrin domain) and a degradative domain (metalloprotease domain). For recent reviews on ADAMs, see Refs 4–7. The focus of this review is on ADAMs 9, 10, 12 and 17. These ADAMs have been shown to possess metalloprotease activity against substrates that either are, or are likely to be, physiologically relevant.

Disintegrin and cytoplasmic domain function

In addition to the pro and metalloprotease domain, all ADAMs contain four domains: disintegrin, cysteine rich, transmembrane and cytoplasmic tail (Fig. 1). We first review what is known about the disintegrin, cysteine-rich, and cytoplasmic tail domains because these domains might modulate the activity, location or substrate specificity of an ADAM metalloprotease.

All ADAMs contain a disintegrin domain and most contain a 14-residue 'disintegrin loop'. (For a detailed description of ADAM disintegrin domains, see Ref. 7.) All ADAM disintegrin loops contain three cysteines, one

1359-6446/01/$ – see front matter PII: S1359-6446(01)01738-X

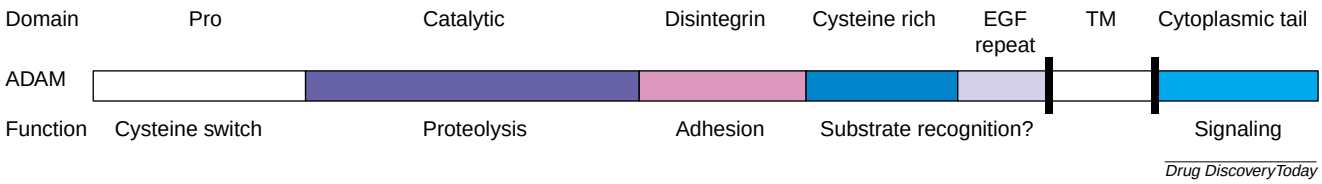

Figure 1. The domain structure of an ADAM (enzymes that contain a disintegrin and a metalloprotease domain) family member. An ADAM family member begins with a pro domain that maintains the enzyme in an inactive state via a cysteine switch mechanism[72,99,100]. The pro domain is also important for proper folding and secretion[101]. The catalytic domain follows the pro domain, is important for proteolysis, and contains the consensus zinc-binding motif, HEXXH. The cysteine-rich region might be important for protein–protein interactions with, for example, substrates or extracellular matrix components, whereas the disintegrin domain has demonstrable binding to integrins. The role of the epidermal growth factor (EGF) repeat is unclear. However, it might be important for substrate recognition. The cytoplasmic tail interacts with SH3 (Src homology) domain-containing proteins and might be important for cell signaling and/or cell cycle control.

each at the beginning, middle and end of the loop. Because the disintegrin domains of the closest ADAM relatives, the snake venom metalloproteases (SVMPs), interact with integrins, it was proposed that ADAM disintegrin domains interact with integrins[8]. Indeed, the disintegrin domains of six ADAMs have been shown to interact with integrins: ADAMS 2, 3 and 9 with $\alpha_6\beta_1$; ADAM 12 with $\alpha_9\beta_1$; ADAM 15 with $\alpha_5\beta_1$, $\alpha_9\beta_1$ and $\alpha_v\beta_3$; and ADAM 23 with $\alpha_v\beta_3$[9–14]. What is not clear is the following: (1) whether or not all ADAM disintegrin domains interact with integrins; (2) whether most disintegrin domains interact with more than one integrin; (3) what defines the integrin specificity (or relative integrin affinities) of various ADAM disintegrin domains; and (4) whether ADAM-integrin interactions are strictly bimolecular. Also, there is only limited information about the sequence requirements of ADAM disintegrin loops. Alanine-scanning mutagenesis has indicated important roles for the aspartic acid immediately following[9], and the glutamine[13] and glutamic acid[10] residues immediately preceding, the central cysteines of the disintegrin loops of mouse ADAMs 2, 3 and 23. The three-amino-acid sequence, RGD, which is uniquely present near the middle of the human ADAM 15 disintegrin loop, is important for interaction with the $\alpha_v\beta_3$ but not with the $\alpha_9\beta_1$ integrin[11,14]. Hence, charged residues near the middle of ADAM disintegrin loops appear to be particularly important.

All ADAMs contain a cysteine-rich region following their disintegrin domains. Although not originally assigned a general function, a recent study has indicated that the cysteine-rich domain of human ADAM 12 can interact with syndecans[15]. Given that integrins and syndecans cooperate in cellular adhesion with the extracellular matrix, a tantalizing possibility is that integrins and syndecans cooperate in ADAM-mediated adhesive events by interacting with ADAM disintegrin and cysteine-rich domains, respectively. An alternate model in which the cysteine-rich domain interacts with a β_1 integrin has also been proposed[15].

All ADAMs have at least one isoform that is predicted to have one transmembrane domain followed by a cytoplasmic tail of varying length, but several ADAMs exist in alternatively spliced forms that either lack transmembrane domains and/or cytoplasmic tails (i.e. are secreted) or that contain alternate cytoplasmic tails. As noted previously[16,17], the cytoplasmic tails of many ADAMs contain sequence homology 3 (SH3) binding sites. Indeed, the cytoplasmic tails of ADAMs 9, 12, 13 and 15 have been shown to interact *in vitro* with Src homology 3 (SH3)-domain-containing proteins including Src family members[18–22]. Recent evidence with *Xenopus* embryos indicates that the interaction between the cytoplasmic tail of *Xenopus* ADAM 13 and the SH3-containing Src family protein PACSIN is biologically relevant[18]. Recent cell biological studies have indicated a functional importance to the interaction between the cytoplasmic tail of ADAM 12 and α-actinin[23]. Hence, it seems probable that ADAMs will interact with intracellular signaling and cytoskeletal systems by virtue of their cytoplasmic tails. Such interactions could dictate the subcellular or plasma membrane microdomain localization of an ADAM protease and hence its accessibility to a physiologically relevant substrate. For example, the cytoplasmic tail of ADAM 9 interacts with protein kinase C, thereby upregulating its cleavage of pro-heparin-binding-epidermal growth factor (HB-EGF)[24]. Furthermore, recent data indicate that the cytoplasmic tail of ADAM 12 influences the amount of ADAM 12 at the cell surface[18,25]. Thus, although the mechanisms are not yet clear, mounting evidence suggests that ADAM cytoplasmic tails can influence ADAM protease function.

Metalloprotease domain function

TACE (ADAM 17)

Many of the processing events that include the release of cytokine factors, shedding of cell surface molecules, release of growth factors and cleavage of amyloid precursor

protein (APP), are all shown to be mediated by metalloproteases. One of the enzymes responsible for some of the metalloprotease-dependent processing events is TACE.

Evidence for TACE as the major physiological TNF-α converting enzyme is compelling. TACE was purified based on its ability to process precursor TNF-α and a peptide that spans the cleavage sequence of the proform of the cytokine at the correct cleavage sequence, and the activity was completely inhibited by a metalloprotease inhibitor of TNF-α release[26,27]. T cells derived from knockout mice that have a disruption in the metalloprotease consensus sequence (HEXXH) found in the exon that encodes TACE ($TACE^{\Delta Zn/\Delta Zn}$), lacks 90% of its ability to process precursor TNF-α (Ref. 26). These results all point to TACE as being the major TNF-α converting enzyme. This is especially important as two groups also identified ADAM 10 (sometimes known as MADM) as a TNF-α converting enzyme[28,29]. However, the k_{cat}/K_m for processing of a peptide substrate that spans the cleavage sequence of precursor TNF-α is tenfold lower for ADAM 10 relative to ADAM 17 (Ref. 30). Also, a potent (<10 nM) inhibitor of ADAM 10, but not of TACE, does not inhibit TNF release from peripheral blood mononuclear cells stimulated with lipopolysaccharide (LPS; M. Moss, unpublished observation), thus providing further evidence for the involvement of TACE as the main TNF-α converting enzyme.

A role for TACE in the processing of other substrates comes from analysis of the phenotype of $TACE^{\Delta Zn/\Delta Zn}$ mice. The $TACE^{\Delta Zn/\Delta Zn}$ mice die between embryonic day 17.5 and the day of birth and exhibit a failure to fuse their eyelids, have thinned corneas, lack a conjunctival sac and have a wavy hair phenotype along with several epidermal defects[31]. This phenotype is characteristic of mice that have a disruption in the TGF-α gene[32,33]. Additional defects identified are epithelial maturation of multiple organs and defects in the spongiotrophoblast layer of the placenta. These defects are reminiscent of mice that have a deletion of the epidermal growth factor receptor (EGFR)[34–36]. The findings point to a general defect in the processing of multiple growth factors of the EGF family of ligands, such as epidermal growth factor (EGF), (HB-EGF), amphiregulin and TGF-α. Such is the case with TGF-α because the growth factor is deficient in fibroblasts taken from the $TACE^{\Delta Zn/\Delta Zn}$ mice that have been immortalized by *ras* transformation[31]. Other putative substrates for TACE that are inhibited by hydroxamic acid inhibitors and that have been identified using the $TACE^{\Delta Zn/\Delta Zn}$ mice are L-selectin[31], TNF receptors I (TNF-RI; Ref. 37) and II (TNF-RII; Ref. 31), APP (Ref. 38), interleukin 1 (IL-1)-receptor II (IL-1RII; Ref. 37), interleukin-6 (IL-6)-receptor[39] and Erb-B4 receptor[40]. A substrate that is inhibited by metalloprotease inhibitors, but of which the secretion is not impaired in the $TACE^{\Delta Zn/\Delta Zn}$ mice, is angiotensin converting enzyme[41]. Finally, TNF-related activation-induced cytokine (TRANCE), an activator of osteoclasts, and the Notch receptor were also recently described as substrates for TACE[42,43].

There is some preliminary *in vitro* biochemical evidence that TACE directly processes some of these substrates. For example, peptides spanning the cleavage sequences of the α-secretase site of APP, L-selectin and the N-terminal processing site of TGF-α are all processed at the correct positions. However, conflicting data comes from inhibitor studies where hydroxamic acids prevent release of TNF-RI and RII with comparable IC_{50} values to TNF secretion, but do not for L-selectin[30] and TGF-α (M. Moss, unpublished observation).

An explanation for the results described with the $TACE^{\Delta Zn/\Delta Zn}$ mice is that the mutant TACE is acting as a dominant-negative by interfering with protein folding and/or some other mechanism in which active TACE is required indirectly for substrate processing. An example of the latter would be a mechanism in which perhaps TACE dimerizes with other disintegrin metalloproteases and indirectly affects their catalytic efficiency. In fact, a dominant-negative form of TACE has been described that retains the disintegrin domain and cytoplasmic tail but lacks the pro and catalytic domains[44]. However, a more full-length $TACE^{\Delta Zn}$ mutant does not prevent the processing of TNF, L-selectin or TNF-RII when cotransfected into COS cells, which indicates that the mutation leads to a loss of function rather than a dominant-negative effect[6]. Experiments with full-length substrates and more comprehensive inhibitor studies might aid in the determination of whether TACE is the physiological convertase for many of the putative substrates identified by the gene knockout studies.

How does TACE distinguish between all of these substrates? Preliminary biochemical data suggests that different constructs of TACE are necessary for turnover of substrate to product. For example, using cells from the $TACE^{\Delta Zn/\Delta Zn}$ mice that are deficient in processing, a chimeric protein encoding the pro and catalytic domains of TACE and downstream domains from ADAM 10 (but lacking an ADAM cytoplasmic tail) was found to be sufficient for cleavage of TNF and TNF RII, whereas the downstream domains of TACE (e.g. disintegrin and cysteine-rich) were also needed to specify IL-1RII shedding[37]. These results suggest that TACE might regulate which substrates it utilizes by existing in different enzyme forms (isolated catalytic versus catalytic–disintegrin–cysteine-rich versus full-length). An alternative mechanism could be that processing of certain substrates is dependent on where

the TACE is localized. Although most of the TACE exists in a perinuclear compartment, it is also present on the cell membrane[26,45]. PMA stimulation downregulates TACE on the cell surface[46], a finding that is consistent with TACE relocalizing to the *trans*-Golgi where it is needed to process substrates such as precursor TNF-α.

Kuz/ADAM 10

ADAM 10 is the first ADAM family member to have a known proteolytic function. ADAM 10 was originally purified from bovine brain based on its ability to cleave myelin basic protein[47]. ADAM 10 has homology to TACE but is most closely related to Kuzbanian (Kuz), its *Drosophila* homolog that has a role in cell-fate determination during neurogenesis and myoblast differentiation[48,49]. ADAM 10 is, therefore, also referred to as mammalian Kuz.

ADAM 10 mRNA is found in bone, bone cells and cartilage[50]. In addition, high levels of ADAM 10 protein are found in the brain[47]. These findings support claims that ADAM 10 has important functions in the brain as well as in cartilage and bone metabolism, whereas the list of substrates for ADAM 10 (Kuz) suggests a variety of roles for this enzyme. So far, ADAM 10 has been shown to process precursor TNF-α[28,29], type IV collagen[51], APP (Ref. 52) and ephrin-A2 (Ref. 53).

There is an increasing body of evidence for the role of ADAM 10 in the processing of APP (Ref. 52). Transfection of HEK 293 cells with ADAM 10 increases the ability of the cells to produce soluble APP-α (sAPP-α). Also, a point mutation in the catalytic domain of ADAM 10 renders it inactive and transfection of this construct into HEK cells acts as a dominant-negative that precludes release of sAPP-α. Finally, ADAM 10 can directly process a substrate that spans the cleavage sequence of APP. Together with the high levels of ADAM 10 found in the brain, these results imply that ADAM 10 is an α-secretase (Fig. 2). It should be noted that TACE is also considered to be an α-secretase as fibroblast cells from TACE knockout mice that are deficient in TACE activity are impaired in their ability to produce and release sAPP-α in response to phorbol ester treatment[38]. In further studies, however, a different effect of three hydroxamic-acid-based inhibitors toward TACE and the α-secretase was observed[54]. Thus, TACE is probably not the major α-secretase, but might have an α-secretase role under inflammatory conditions including those that have been associated with Alzheimer's disease.

Given that ADAM 10 might be an α-secretase for APP processing, it can be considered to be a protective factor in the etiology of Alzheimer's disease as it might funnel products towards the non-amyloidogenic pathway[55–57]. The amyloidogenic and presumed pathological pathway involves cleavage of APP by both β-secretase (BACE) and a γ-secretase–presenilin-1 complex that results in the

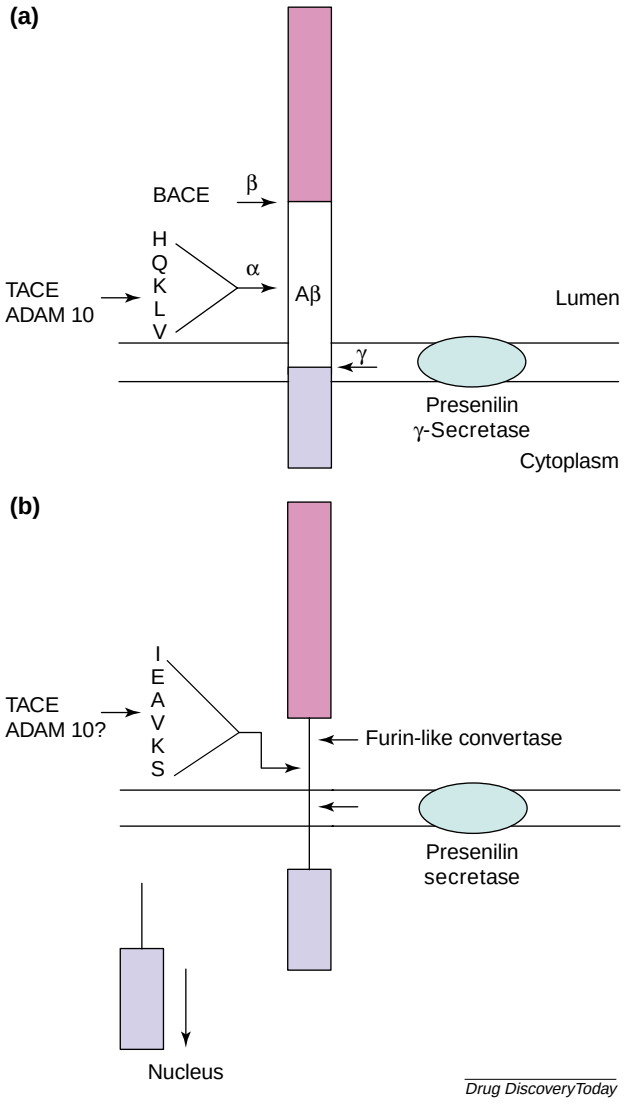

Figure 2. (a) Processing of amyloid precursor protein (APP). APP is processed by α-, β- and γ-secretases. In the non-amyloidogenic pathway, proposed α-secretases, such as ADAM 10 and tumor necrosis factor-α-converting enzyme (TACE), process APP at the α-site. Cleavage of APP at the α-site is thought to preclude cleavage at the β- and γ-sites. For the amyloidogenic pathway, processing of APP occurs at the β-site by BACE[102–104] and at the γ-site by the γ-secretase/presenilin complex or presenilin directly. **(b)** Processing of Notch. A furin-like enzyme is involved in the first cleavage event of Notch. This event is thought to be followed by cleavage between Ala1710 and Val1711 by TACE and/or Kuz (ADAM 10). The intermediate product that is generated is then processed further by another secretase in a presenilin dependent process or by presenilin directly. The cytoplasmic tail is released where it translocates to the nucleus to interact with transcription factors and is involved in cell signaling. Abbreviation: BACE, amyloid precursor protein β-secretase.

formation of Abeta (Fig. 2), which might then aggregate and deposit as extracellular amyloid plaques in the Alzheimer's diseased brain (recently reviewed, Ref. 58).

As in APP processing, Notch protein is thought to be processed in three places; the first event is mediated by a furin-like convertase, the second event might be carried out by Kuz and/or TACE, and the third by a presenilin-dependent process (Fig. 2). For reviews see Refs 6, 59 and 60.

Notch is a transmembrane protein that was named after a *Drosophila* mutant that had notches at the wing margins. Notch is important for cell fate 'decisions' during development. Although Notch was only recently shown to be directly cleaved by TACE, genetic and gene knockout studies implicate Kuz as the actual Notch convertase[61,62]. In genetic screens for neurogenic genes in *Drosophila*, for example, Kuz was identified as a potential mediator of Notch signaling and it was subsequently proposed that Notch was processed by Kuz on ligand binding. Evidence for a role of Kuz in directly processing Notch comes from the phenotype of mice lacking Kuz because it resembles the phenotype of mice lacking Notch (Ref. 61).

Similar to Notch, biochemical data exists for a role of the *Drosophila* Kuz in processing of the ligand for Notch, delta. Overexpression of Kuz in cells also expressing delta increases the processing of delta. In addition, a dominant-negative form of Kuz inhibits processing. However, delta has not yet been shown to be directly cleaved by Kuz, so more biochemical data is needed to confirm the findings by Qi and coworkers[63].

Recently, another role for ADAM 10 in the brain has been the result of studies in which the enzyme is involved in the processing of ephrin-A2 (Ref. 53), a well characterized axon repellant. A membrane form of ephrin-A2 exists that binds the ephrin-A3 receptor. After binding to its receptor, ephrin-A2 is processed by ADAM 10 to generate a soluble form. Given that the soluble truncated form cannot activate the receptor, the cleavage process results in axon detachment and, probably, termination of signaling. As in the studies undertaken with APP, a dominant-negative version of ADAM 10 was generated. This construct lacked the protease domain and could inhibit ectodomain shedding of ephrin-A2. Cleavage mediated by ADAM 10 was shown to be important for axon guidance because a cleavage-inhibiting mutation in ephrin-A2 delayed axon withdrawal. Interestingly, the *Drosophila* homolog of ADAM 10, Kuz, was identified in a *Drosophila* genetic screen as being required for normal axon extension[48].

ADAM 12 (Meltrin-α)

ADAM 12 or Meltrin-α was identified and cloned from a mouse myogenic cell line by Fujisawa-Sehara and colleagues[64]. ADAM 12, originally found in neonatal skeletal muscle and bone, has subsequently been identified in placenta, tumor cell lines, normal human adult skeletal, cardiac and smooth muscle tissues. A soluble form, ADAM 12-S, and a long, membrane-bound form, ADAM 12-L arise from alternative splicing[65].

The first evidence for the involvement of ADAM 12 in myoblast fusion came from studies showing that transfection of mouse C2 cells with a minigene for ADAM 12 lacking the pro and metalloprotease domains accelerated cell fusion. A construct containing the full-length ADAM 12 inhibited myoblast fusion, implying that fusion is regulated through proteolysis of some unknown factor by the metalloprotease domain[64].

Recently, ADAM 12-S has demonstrated proteolytic activity[66]. Initially, an α-2-macroglobulin assay was utilized. This assay relies on covalent modification of the α-2-macroglobulin by an active protease. Shifts in molecular weight are then visualized using western analysis. A substrate for ADAM 12-S, insulin-like growth factor-binding protein 3 (IGFBP-3), was then identified using a yeast two-hybrid system in which placental proteins were screened for their ability to interact with IGFBP-3 (Ref. 67). This finding has broad implications bcause IGFBP-3 binds the majority of insulin growth factor I and II (IGF I and II) with equal or greater affinity than the IGF receptors. IGFBP-3 is thought to function by sequestering IGF I or II until it is released by proteolysis of the binding protein via ADAM 12-S and/or other processing events. The balance between the IGFs and the IGFBPs is crucial for osteoarthritis and diabetes with which decreased IGF levels are associated[68,69]. Experiments are just beginning to unravel the role of IGFBP-3 and its proteolytic fragments[70].

ADAM 9 (MDC 9; meltrin-γ)

ADAM 9, also known as MDC 9 or meltrin-γ, was characterized by Blobel and colleagues[22] and is expressed in a variety of tissues and in hematological malignancies[71]. Substrate studies have begun to unravel possible roles for ADAM 9. At about the same time, two papers were published that describe substrates for ADAM 9. The first was by Izumi and coworkers[24] and demonstrates that ADAM 9 might process the EGFR ligand, HB-EGF, but no direct evidence is presented. However, overexpression of full-length ADAM 9 increases HB-EGF shedding. Dominant-negative effects were observed by ADAM 9 mutants that had mutations in the active-site HEXXH motif (chelators of zinc). These results further implicate ADAM 9 as a processing enzyme for HB-EGF, although improper folding and transport to the cell surface might be impaired in these mutants.

The second paper, by Rhogani and colleagues[72], shows direct evidence that ADAM 9 can process a variety of substrates with good specificity constants. A peptide that spans the cleavage sequence of APP is processed by ADAM 9, except that the cleavage position is distinct from the normal site that generates APP-α. The authors provide evidence for the first time that ADAM 9 might have a substrate specificity that is comparable to the matrix metalloproteases (MMPs). A classical MMP substrate is processed with a k_{cat}/K_m that is similar to that found for the MMPs at the MMP-specific cleavage site. Also, a peptide based on the cleavage sequence of precursor TNF-α is cleaved at sites similar to those found for MMPs such as gelatinase B (M. Moss, unpublished observation). The fact that ADAM 9 has similar substrate requirements to the MMPs suggests that this enzyme might also degrade different types of collagen, a finding that is consistent with the ability of some SVMPs to process extracellular matrix proteins. Unlike many of the other ADAMs, where much of the protein is found in a perinuclear compartment such as the *trans*-Golgi, ADAM 9 is localized to the cell surface[22] where it can degrade matrix components such as collagen.

Structural features and inhibitors of TACE

The structure of the catalytic domain of TACE, bound to a hydroxamate inhibitor, has been determined using X-ray crystallography[73]. To date, this is the only mammalian ADAM structure reported. However, several closely related SVMP structures have been solved, including adamalysin II (Ref. 74) and atrolysin C (Ref. 75). Within the catalytic domain, the TACE sequence is ~35% identical to ADAM 10, but only 20–25% identical to most of the other ADAMs. By contrast, the adamalysin and atrolysin sequences are 20–25% identical to TACE and ADAM 10 but 37–41% identical to ADAM 9 and ADAM 12. This suggests that the TACE structure is a good model for ADAM 10, whereas the SVMP structures serve as better models for ADAM 9 and ADAM 12.

The overall structures of TACE, adamalysin II and atrolysin C are similar, with α-helices packed above and below a central β-sheet. This fold is generally similar to that of the MMP family, although many of the peripheral loops have different conformations. The structures are especially similar around the catalytic site, in which TACE, adamalysin and the MMPs all bind zinc with the same geometry. In all cases, the active site cleft is deeper on the 'primed' (right-hand) side of the zinc, corresponding to the P1′, P2′ and P3′ residues of the substrate, than on the 'unprimed' (left-hand) side, corresponding to the P1, P2 and P3 residues. The S1′ pocket is the deepest and it appears to be especially large and deep in adamalysin and atrolysin[76].

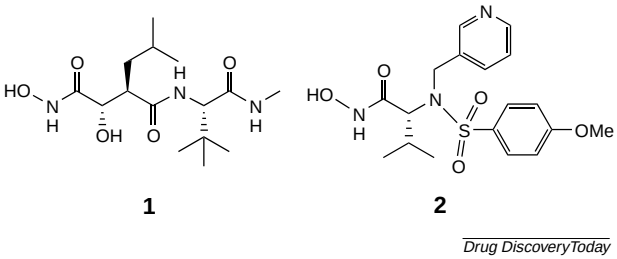

Figure 3. Prototypical matrix metalloproteases (MMPs) and tumor necrosis factor-α-converting enzyme (TACE) inhibitors, marimastat **1** and CGS27023 **2**.

The S1′ pocket is somewhat shallower in TACE and has a different shape that connects below Leu384 and Ala439 with the S3′ pocket[73]. The TACE, adamalysin, atrolysin and MMP structures have been solved with various different inhibitors. In most cases, the inhibitors contain a 'headgroup,' such as hydroxamate, that ligates the zinc, a peptide or peptide-mimetic backbone that lies in the 'primed' (right-hand) side of the active-site cleft, and a lipophilic substituent directed into the S1′ pocket[77–79].

To date, TACE inhibitors reported in the literature are almost exclusively of the 'right-hand-side design' previously described for MMP inhibitors[80] and are of either the succinyl hydroxamate (exemplified by **1** in Fig. 3) or the right-hand side peptidomimetic/sulfonamide hydroxamate variety (exemplified by **2** in Fig. 3)[81].

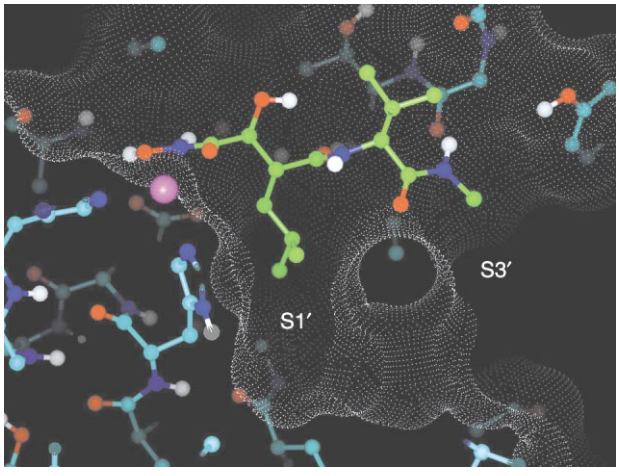

Figure 4. Model of marimastat bound to human tumor necrosis factor-α-converting enzyme (TACE). Hydrogen, oxygen, nitrogen and zinc atoms are represented in white, red, dark blue and magenta, respectively. Carbon atoms are blue and green in the protein and the inhibitor, respectively. The surface of the protein is depicted with a Connolly dot surface.

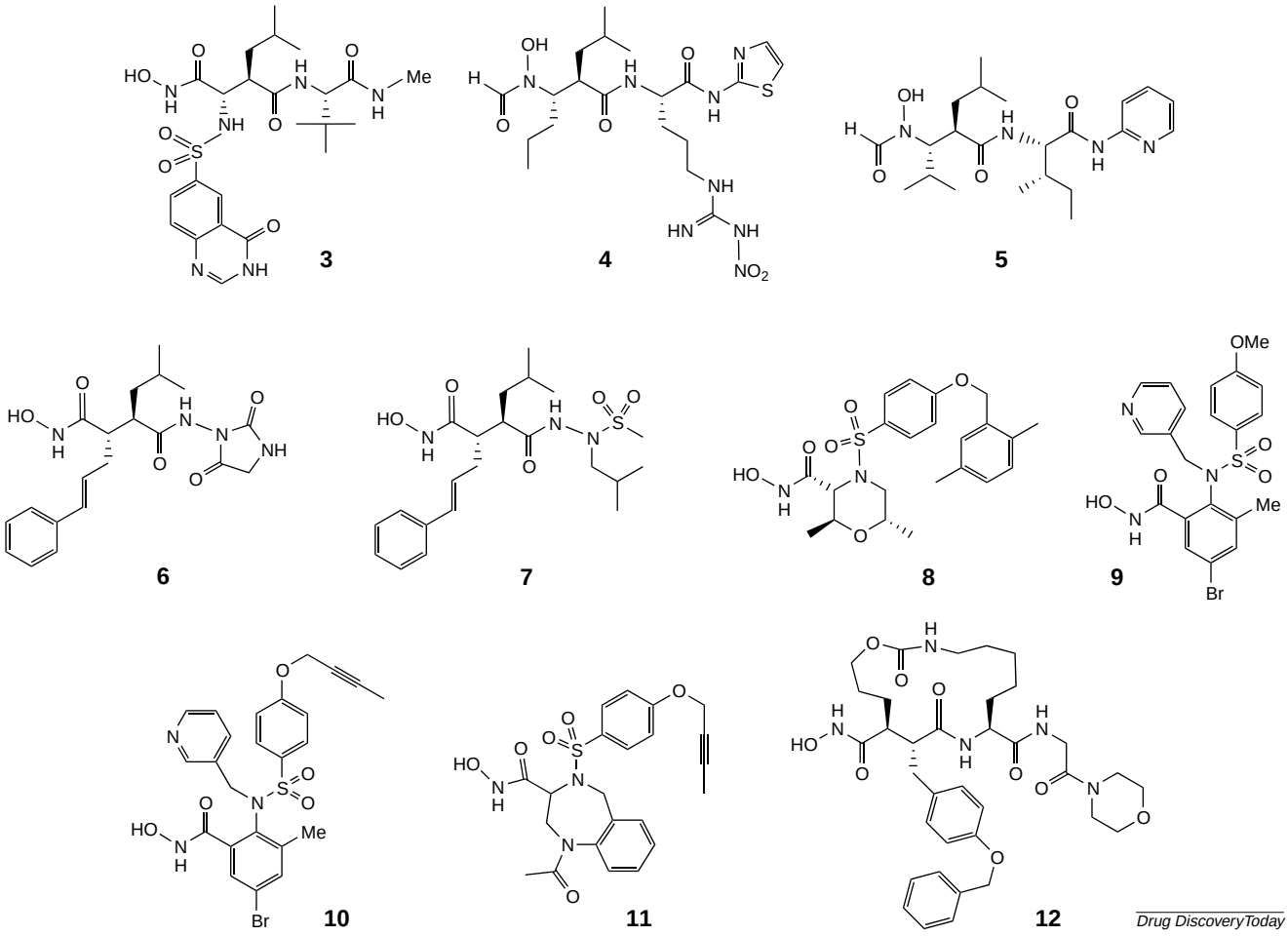

Figure 5. Recently disclosed tumor necrosis factor-α-converting enzyme (TACE) inhibitors.

The hydroxamate BB2516 (marimastat **1**) is potent against TACE, ADAM 9 and MMPs (Ref. 72). Marimastat and the sulfonamide CGS27023 (Ref. 82; **2**), also an inhibitor of cell-free TACE, cellular TNF secretion[83] and ADAM 9, continue to serve as basic templates for the design of TACE inhibitors disclosed in the literature. We have docked marimastat into TACE using the MVP program[84] as shown in Fig. 4. The P1′ isobutyl group is directed into the S1′ pocket, but does not fill it, which suggests that stronger affinity could be obtained with larger P1′ substituents. Larger groups could also be accommodated at P3′ giving an increase in potency for inhibition of TNF release[85]. The molecules in Fig. 5 have been disclosed in recent reviews of TACE inhibitors[86]. Derivatization of the position alpha to the hydroxamate in the succinate inhibitor template led to potent inhibitors of TACE having submicromolar IC_{50} values for inhibition of TNF release in human whole blood[87]. The best compound, (**3**; Fig. 5), possesses a K_i value of 0.57 nM against TACE and an IC_{50} value of 280 nM against TNF in human whole blood.

The *N*-hydroxyformamide, or 'reverse hydroxamate' functional group, can serve to bind to zinc in MMPs and thus has been explored as a zinc chelator in TACE and MMP inhibitors. The synthesis and biological activity of the *N*-hydroxyformamide GW4459 (**4**; Fig. 5) has been described[88]. This molecule is a potent inhibitor of TACE (K_i = 4 nM) and MMPs, and inhibits TNF release from cultured MonoMac-6 cells (IC_{50} = 34 nM). Further, GW4459 administered subcutaneously reduces TNF levels in the pleural fluid of zymosan-treated rats. Related compounds were synthesized possessing a methyl substitution at the P2′ arginine side-chain vicinal to the peptide backbone, with the aim of endowing the compound with stability and oral bioavailability.

An example of branched methyl substitution at P2′ in the *N*-hydroxyformamide inhibitor class is GW3333 (**5**; Fig. 5), which is an inhibitor of TACE and the MMPs. Furthermore, the compound is orally active with a long half-life in rat and dog. The compound effectively inhibits TNF release in mice subjected to LPS administration[85].

TACE inhibitors have been designed that possess P2′ amino amide mimetics or truncated P2′-P3′ substituents. The cyclic hydrazide (**6**; Fig. 5) is a potent inhibitor of TNF release from THP-1 cells (IC_{50} = 268 nM; Ref. 89). Although no data on direct TACE enzyme inhibition are available, **6** is similar to Ro327315 (**7**; Fig. 5), which inhibits TACE (K_i = 3 nM) and TNF release from THP-1 cells (IC_{50} = 375 nM; Ref. 90) and has entered phase I clinical trials.

The morpholine derivative (**8**; Fig. 5) is a P1-P2′ constrained analog of 2 and is reported to inhibit TACE with a K_i value of 10 nM (Ref. 91). Compound **8** is selective against collagenase-1 (K_i = 3500 nM) and inhibits collagenase-3 with a K_i value of 3 nM. Despite the presence of three asymmetric centers the morpholine ring of **8** and its congeners are easily synthesized from threonine.

The elaboration of anthranilic acid and related bicyclic aromatic skeletons into TACE and MMP inhibitors is an example of substantial innovation in TACE/MMP inhibitor design[92]. The anthranilic hydroxamate (**9**; Fig. 5) inhibits TACE with a K_i value of 40 nM. Remarkably, adjustment of the arylsulfenyl substituent from methoxy to 2-butynyloxy produces compound **10** (Fig. 5) with a K_i value of 15 nM against TACE and concurrent selectivity against collagenase-1 and collagenase-3. The butynyloxy substituent binds well in the angled S1′ subsite of TACE (Ref. 73). Such a modification on the benzazepine **11** (Fig. 5) has the same effect, producing a molecule with a K_i value of 16 nM against TACE and selectivity against collagenase-1.

Certain succinyl hydroxamates, when bridged between P1 and P2′, afford potent MMP/TACE inhbitors[93]. The macrocyclic carbamate **12** possesses K_i values of 18 nM and 5000 nM against TACE and collagenase-1, respectively. Members of this inhibitor class are orally bioavailable and reduce TNF titer *in vivo*.

Conclusion

The immense therapeutic potential for small-molecule TNF inhibitors has driven an intense search for metalloprotease inhibitors that are selective and potent for TACE and inhibit TNF release from cell lines. The application of pharmaceutical industry resources has resulted in a multitude of patent applications and open disclosures of molecules inhibiting TACE and TNF release from stimulated cell preparations. That the ADAMs 9, 10 and 12 have similarities to TACE and the MMPs implies that many of the inhibitors in the literature will also prove to be potent against other family members. Although inhibition of TACE might be useful in any disease state where TNF antagonism has been validated, such as arthritis[94], diabetes[95], HIV cachexia[96], sepsis[97] and cancer[98], activation of this ADAM and/or ADAM 10 might also prove useful for the treatment of Alzheimer's disease. Applications of ADAM 9 inhibitors at this time are unclear, as is the potential effect an ADAM 12 inhibitor would have in disease states such as osteoarthritis, cancer and diabetes. The future promises exciting developments for the ADAMs and the inhibitors of this family of enzymes.

Acknowledgement

We thank Carl Blobel for his careful reading of the manuscript.

References

1 Wolfsberg, T.G. *et al.* (1995) ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. *Dev. Biol.* 169, 378–383
2 Kaushal, G.P. and Shah, S.V. (2000) The new kids on the block: ADAMTSs, potentially multifunctional metalloproteinases of the ADAM family. *J. Clin. Invest.* 105, 1335–1337
3 Cerretti, D.P. *et al.* (1999) Isolation of two novel metalloproteinase-disintegrin (ADAM) cDNAs that show testis-specific gene expression. *Biochem. Biophys. Res. Commun.* 263, 810–815
4 Black, R.A. and White, J.M. (1998) ADAMs: focus on the protease domain. *Curr. Opin. Cell Biol.* 10, 654–659
5 Primakoff, P. and Myles, D.G. (2000) The ADAM gene family: surface proteins with adhesion and protease activity. *Trends Genet.* 16, 83–87
6 Schlondorff, J. and Blobel, C.P. (1999) Metalloprotease-disintegrins: modular proteins capable of promoting cell–cell interactions and triggering signals by protein-ectodomain shedding. *J. Cell Sci.* 112, 3603–3617
7 White, J.M. *et al.* ADAMs. In *Cell Adhesion: Frontiers in Molecular Biology* (Beckerle, M., ed.), Oxford University Press (in press)
8 Blobel, C.P. *et al.* (1992) A potential fusion peptide and an integrin ligand domain in a protein active in sperm–egg fusion. *Nature* 356, 248–252
9 Bigler, D. *et al.* (2000) Sequence-specific interaction between the disintegrin domain of mouse ADAM 2 (fertilin β) and murine eggs: role of the α6 integrin subunit. *J. Biol. Chem.* 275, 11576–11584
10 Cal, S. *et al.* (2000) ADAM 23/MDC3, a human disintegrin that promotes cell adhesion via interaction with the $\alpha_v\beta_3$ integrin through an RGD-independent mechanism. *Mol. Biol. Cell* 11, 1457–1469
11 Eto, K. *et al.* (2000) RGD-independent binding of integrin $\alpha_9\beta_1$ to the ADAM 12 and -15 disintegrin domains mediates cell–cell interaction. *J. Biol. Chem.* 275, 34922–34930
12 Nath, D. *et al.* (2000) Meltrin γ (ADAM-9) mediates cellular adhesion through $\alpha_6\beta_1$ integrin, leading to a marked induction of fibroblast cell motility. *J. Cell Sci.* 113, 2319–2328
13 Takahashi, Y. *et al.* Sequence-specific interaction between the disintegrin domain of mouse ADAM 3 and murine eggs: role of the β1 integrin associated proteins CD9, CD81 and CD98. *Mol. Biol. Cell* (in press)
14 Zhang, X-P. *et al.* (1998) Specific interaction of the recombinant disintegrin-like domain of MDC-15 (Metargidin, ADAM-15) with integrin $\alpha_v\beta_3$. *J. Biol. Chem.* 273, 7345–7350
15 Iba, K. *et al.* (2000) The cysteine-rich domain of human ADAM 12 supports cell adhesion through syndecans and triggers signaling events that lead to β1 integrin-dependent cell spreading. *J. Cell Biol.* 149, 1143–1156
16 Wolfsberg, T.G. *et al.* (1995) ADAM, a novel family of membrane proteins containing A disintegrin and metalloprotease domain: multipotential functions in cell–cell and cell–matrix interactions. *J. Cell Biol.* 131, 275–278
17 Wolfsberg, T.G. and White, J.M. (1996) ADAMs in fertilization and development. *Dev. Biol.* 180, 389–401

18 Cousin, H. *et al*. (2000) PACSIN2 is a regulator of the metalloprotease/disintegrin ADAM13. *Dev. Biol*. 227, 197–210

19 Howard, L. *et al*. (1999) Interaction of the metalloprotease disintegrins MDC9 and MDC15 with two SH3 domain-containing proteins, endophilin I and SH3PX1. *J. Biol. Chem*. 274, 31693–31699

20 Kang, Q. *et al*. (2000) Metalloprotease-disintegrin ADAM 12 binds to the SH3 domain of Src and activates Src tyrosine kinase in C2C12 cells. *Biochem. J*. 352, 883–892

21 Nelson, K.K. *et al*. (1999) Evidence for an interaction of the metalloprotease-disintegrin tumour necrosis factor α convertase (TACE) with mitotic arrest deficient 2 (MAD2), and of the metalloprotease-disintegrin MDC9 with a novel MAD2-related protein, MAD2β. *Biochem. J*. 343, 673–680

22 Weskamp, G. *et al*. (1996) MDC9, a widely expressed cellular disintegrin containing cytoplasmic SH3 ligand domains. *J. Cell Biol*. 132, 717–726

23 Galliano, M.F. *et al*. (2000) Binding of ADAM 12, a marker of skeletal muscle regeneration, to the muscle-specific actin-binding protein, α-actinin-2, is required for myoblast fusion. *J. Biol. Chem*. 275, 13933–13939

24 Izumi, Y. *et al*. (1998) A metalloprotease-disintegrin, MDC9/meltrin-γ/ADAM 9 and PKCδ are involved in TPA-induced ectodomain shedding of membrane anchored heparin-binding EGF-like growth factor. *EMBO J*. 17, 7260–7272

25 Hougaard, S. *et al*. (2000) Trafficking of human ADAM 12-L: retention in the *trans*-Golgi network. *Biochem. Biophys. Res. Commun*. 275, 261–267

26 Black, R.A. *et al*. (1997) A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. *Nature* 385, 729–732

27 Moss, M.L. *et al*. (1997) Cloning of a disintegrin metalloproteinase that processes precursor tumor-necrosis factor-α. *Nature* 385, 733

28 Rosendahl, M.S. *et al*. (1997) Identification and characterization of a pro-tumor necrosis factor-α-processing enzyme from the ADAM family of zinc metalloproteases. *J. Biol. Chem*. 272, 24588–24593

29 Lunn, C.A. *et al*. (1997) Purification of ADAM 10 from bovine spleen as a TNF-α convertase. *FEBS Lett*. 400, 333–335

30 Moss, M.L. *et al*. (1999) TNF convertase. In *Metalloproteinases as Targets for Anti-Inflammatory Drugs* (Bradshaw, D. *et al*., eds), pp.187–203, Birkhäuser Publishing

31 Peschon, J.J. *et al*. (1998) An essential role for ectodomain shedding in mammalian development. *Science* 282, 1281–1284

32 Luetteke, N.C. *et al*. (1993) TGF-α deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. *Cell* 73, 263–278

33 Mann, G.B. *et al*. (1993) Mice with a null mutation of the TGF-α gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. *Cell* 73, 249–261

34 Miettin, P.J. *et al*. (1995) Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* 376, 337–341

35 Threadgill, D.W. *et al*. (1995) Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science* 269, 230–234

36 Sibilia, M. and Wagner, E.F. (1995) Strain-dependent epithelial defects in mice lacking the EGF receptor. *Science* 269, 234–238

37 Reddy, P. *et al*. (2000) Functional analysis of the domain structure of tumor necrosis factor-α converting enzyme. *J. Biol. Chem*. 275, 14608–14614

38 Buxbaum, J.D. *et al*. (1998) Evidence that tumor necrosis factor-α converting enzyme is involved in regulated α-secretase cleavage of the Alzheimer amyloid protein precursor. *J. Biol. Chem*. 273, 27765–27767

39 Althoff, K. *et al*. (2000) Shedding of interleukin-6 receptor and tumor necrosis factor α. Contribution of the stalk sequence to the cleavage pattern of transmembrane proteins. *Eur. J. Biochem*. 267, 2624–2631

40 Rio, C. *et al*. (2000) Tumor necrosis factor-α-converting enzyme is required for cleavage of erbB4/HER4. *J. Biol. Chem*. 275, 10379–10387

41 Sadhukhan, R. *et al*. (1999) Unaltered cleavage and secretion of angiotensin-converting enzyme in tumor necrosis factor-α-converting enzyme-deficient mice. *J. Biol. Chem*. 274, 10511–10516

42 Lum, L. *et al*. (1999) Evidence for the role of tumour necrosis factor-α (TNF-α)-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. *J. Biol. Chem*. 274, 13613–13618

43 Brou, C. *et al*. (2000) A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. *Mol. Cell* 5, 207–216

44 Solomon, K.A. *et al*. (1999) Cutting edge: a dominant-negative form of TNF-α converting enzyme inhibits proTNF and TNFRII secretion. *J. Immunol*. 163, 4105–4108

45 Schlondorff, J. *et al*. (2000) Intracellular maturation and localization of the tumour necrosis factor-α convertase (TACE). *Biochem. J*. 347, 131–138

46 Doedens, J.R. *et al*. (2000) Stimulation-induced downregulation of tumor necrosis factor-α converting enzyme. *J. Biol. Chem*. 275, 14598–14607

47 Howard, L. *et al*. (1996) Molecular cloning of MADM: a catalytically active mammalian disintegrin-metalloprotease expressed in various cell types. *Biochem. J*. 317, 45–50

48 Fambrough, D. *et al*. (1996) The cell surface metalloprotease/disintegrin Kuzbanian is required for axonal extension in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A*. 93, 13233–13238

49 Rooke, J. *et al*. (1996) KUZ, a conserved metalloprotease-disintegrin protein with two roles in *Drosophila* neurogenesis. *Science* 273, 1227–1231

50 Dallas, D.J. *et al*. (1999) Localization of ADAM 10 and Notch receptors in bone. *Bone* 25, 9–15

51 Millichip, M.I. *et al*. (1998) The metallo-disintegrin ADAM 10 (MADM) from bovine kidney has type IV collagenase activity *in vitro*. *Biochem. Biophys. Res. Commun*. 245, 594–598

52 Lammich, S. *et al*. (1999) Constitutive and regulated α-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc. Natl. Acad. Sci. U. S. A*. 96, 3922–3927

53 Hattori, M. *et al*. (2000) Regulated cleavage of a contact-mediated axon repellent. *Science* 289, 1360–1365

54 Parvathy, S. *et al*. (1998) The secretases that cleave angiotensin converting enzyme and the amyloid precursor protein are distinct from tumour necrosis factor-α convertase. *FEBS Lett*. 431, 63–65

55 Skovronsky, D.M. *et al*. (2000) Protein kinase C-dependent α-secretase competes with β-secretase for cleavage of amyloid-β precursor protein in the *trans*-Golgi network. *J. Biol. Chem*. 275, 2568–2575

56 Esch, F.S. *et al*. (1990) Cleavage of amyloid β peptide during constitutive processing of its precursor. *Science* 248, 1122–1124

57 Sisodia, S. *et al*. (1990) Evidence that β-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science* 248, 492–495

58 Nunan, J. and Small, D.H. (2000) Regulation of APP cleavage by α-, β- and γ-secretases. *FEBS Lett*. 483, 6–10

59 Kopan, R. and Goate, A. (2000) A common enzyme connects Notch signaling and Alzheimer's disease. *Genes Dev*. 14, 2799–2806

60 Zhang, Z. *et al*. (2000) Presenilins are required for γ-secretase cleavage of β-APP and transmembrane cleavage of Notch-1. *Nat. Cell Biol*. 2, 463–465

61 Mumm, J.S. *et al*. (2000) A ligand-induced extracellular cleavage regulates γ-secretase-like proteolytic activation of Notch 1. *Mol. Cell* 5, 197–206

62 Pan, D. and Rubin, G.M. (1997) Kuzbanian controls proteolytic processing of Notch and mediates lateral inhibition during *Drosophila* and vertebrate neurogenesis. *Cell* 90, 271–280

63 Qi, H. *et al*. (1999) Processing of the Notch ligand delta by the metalloprotease Kuzbanian. *Science* 283, 91–94

64 Yagami-Hiromasa, T.A. *et al*. (1995) A metalloprotease-disintegrin participating in myoblast fusion. *Nature* 377, 652–656

65 Gilpin, B.J. *et al*. (1998) A novel, secreted form of human ADAM 12 (meltrin α) provokes myogenesis *in vivo*. *J. Biol. Chem*. 273, 157–166

66 Loechel, F. *et al*. (1998) Human ADAM 12 (meltrin α) is an active metalloprotease. *J. Biol. Chem*. 273, 16993–16997

67 Shi, Z. *et al.* (2000) ADAM 12, a disintegrin metalloprotease, interacts with insulin-like growth factor-binding protein-3. *J. Biol. Chem.* 275, 18574–18580

68 Martel-Pelletier, J. *et al.* (1998) IGF/IGFBP axis in cartilage and bone in osteoarthritis pathogenesis. *Inflamm. Res.* 47, 90–100

69 Wetterau, L.A. *et al.* (1999) Novel aspects of the insulin-like growth factor binding proteins. *Mol. Genet. Metab.* 68, 161–181

70 Devi, G.R. *et al.* (2000) Differential effects of insulin-like growth factor (IGF)-binding protein-3 and its proteolytic fragments on ligand binding, cell surface association, and IGF-I receptor signaling. *Endocrinology* 141, 4171–4179

71 Wu, E. *et al.* (1997) Expression of members of the novel membrane linked metalloproteinase family ADAM in cells derived from a range of haematological malignancies. *Biochem. Biophys. Res. Commun.* 235, 437–442

72 Roghani, M. *et al.* (1999) Metalloprotease-disintegrin MDC9: intracellular maturation and catalytic activity. *J. Biol. Chem.* 274, 3531–3540

73 Maskos, K. *et al.* (1998) Crystal structure of the catalytic domain of human tumor necrosis factor-α-converting enzyme. *Proc. Natl. Acad. Sci. U. S. A.* 95, 3408–3412

74 Gomis-Ruth, F.X. *et al.* (1994) Refined 2.0 Å X-ray crystal structure of the snake venom zinc endopeptidase adamalysin II. Primary and tertiary structure determination, refinement, molecular structure and comparison with astacin, collagenase and thermolysin. *J. Mol. Biol.* 239, 513–544

75 Zhang, D. *et al.* (1994) Structural interaction of natural and synthetic inhibitors with the venom metalloproteinase, atrolysin C (form d). *Proc. Natl. Acad. Sci. U. S. A.* 91, 8447–8451

76 Botos, I. *et al.* (1996) Batimastat, a potent matrix metalloproteinase inhibitor, exhibits an unexpected mode of binding. *Proc. Natl. Acad. Sci. U. S. A.* 93, 2749–2754

77 Cirilli, M. *et al.* (1997) 2 Å X-ray structure of adamalysin II complexed with a peptide phosphonate inhibitor adopting a retro-binding mode. *FEBS Lett.* 418, 319–322

78 Gomis-Ruth, F. *et al.* (1998) Structures of adamalysin II with peptidic inhibitors. Implications for the design of tumor necrosis factor-α convertase inhibitors. *Protein Sci.* 7, 283–292

79 Gonnella, N.C. *et al.* (1997) Bioactive conformation of a potent stromelysin inhibitor determined by X-nucleus filtered and multidimensional NMR spectroscopy. *Bioorg. Med. Chem.* 5, 2193–2201

80 Beckett, R.P. *et al.* (1996) Recent advances in matrix metalloproteinase inhibitor research. *Drug Discov. Today* 1, 16–26

81 Beckett, R.P. and Whittaker, M. (1998) Matrix metalloproteinase inhibitors. *Expert Opin. Ther. Pat.* 8, 259–282

82 Parker, D.T. *et al.* (1994) The development of 27023A: a novel, potent, and orally active matrix metalloprotease inhibitor. *Fourth International Conference, Inflammation Research Association, October 1994. White Haven, PA, USA*; p73 (Abstracts)

83 Barberia, J.T. *et al.* (1996) Development of a cell-based assay for TNF-α release which avoids transcription and translation steps. *Fifth International Conference, Inflammation Research Association, October 1996. Hershey, PA, USA*; p1 (Abstracts)

84 Lambert, M.H. (1997) Docking conformationally flexible molecules into protein binding sites. In *Practical Application of Computer-Aided Drug Design* (Charifson, P.S., ed.), pp. 243–303, Marcel-Dekker

85 Moss, M.L. *et al.* (2000) Screening and design of tumor necrosis factor-α converting enzyme inhibitors. In *Cytokine Inhibitors* (Ciliberto, G. and Savino, R., eds), pp. 163–176, Marcel Dekker

86 Becherer, J.D. *et al.* (2000) The tumor necrosis factor-α converting enzyme. In *Proteases as Targets for Therapy*: *Handbook of Experimental Pharmacology* (von der Helm, K. *et al.*, eds), (Vol. 140), pp. 235–258, Springer-Verlag

87 Barlaam, B. *et al.* (1999) New α-substituted succinate-based hydroxamic acids as TNF-α convertase inhibitors**.** *J. Med. Chem.* 42, 4890–4908

88 Rabinowitz, M. *et al.* (2000) Synthesis and evaluation of novel *N*-hydroxy formamide inhibitors of tumor necrosis factor-α converting enzyme. *220th National Meeting of the American Chemical Society, Washington DC, August 2000, American Chemical Society, Washington DC*

89 Broadhurst, M.J. *et al.* (2000) Cyclic hydrazine derivatives as TNF-α inhibitors. *PCT Int. Appl.* WO 0035885 A1

90 Nixon, J.S. (1999) Design of metalloproteinase inhibitors for treatment of arthritis. *TNF: A Validated Target with Multiple Therapeutic Potential, Second International Meeting, Princeton, NJ*; Strategic Research Institute, New York, NY

91 McClure, K.F. *et al.* (2000) Preparation of 4-phenylsulfonyl-3-morpholinehydroxamic acids and analogs as tumor necrosis factor-α-convertase inhibitors. *PCT Int. Appl.* WO 0009492 A1

92 Skotkicki, J. *et al.* (2000) Anthranilic acid and benzodiazepine sulfonamides as novel MMP inhibitors. *International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December 2000*; MEDI-168

93 Decicco, C.P. (2000) Rational design of peptidomimetic inhibitors of TNF-α processing aggrecanase. *International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December 2000*; MEDI-212

94 DiMartino, M. *et al.* (1997) Anti-arthritic activity of hydroxamic acid-based pseudopeptide inhibitors of matrix metalloproteinases and TNF-α processing. *Inflammation Res.* 46, 211–215

95 Morimoto, Y. *et al.* (1997) KBR7785, a novel matrix metalloproteinase inhibitor, exerts its antidiabetic effect by inhibiting tumor necrosis factor-α production. *Life Sci.* 61, 795–803

96 Belec, L. *et al.* (1995) Increased tumor necrosis factor-α serum levels in patients with HIV wasting syndrome and euthyroid sick syndrome. *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* 8, 212–214

97 Mohler, K.M. *et al.* (1994) Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. *Nature* 370, 218–220

98 Drummond, A.H. *et al.* (1999) Preclinical and clinical studies of MMP inhibitors in cancer. *Ann. New York Acad. Sci.* 878, 228–235

99 Loechel, F. *et al.* (1999) Regulation of human ADAM 12 protease by the prodomain. Evidence for a functional cysteine switch. *J. Biol. Chem.* 274, 13427–13433

100 Van Wart, H.E. and Birkedal-Hansen, H. (1990) The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc. Natl. Acad. Sci. U. S. A.* 87, 5578–5582

101 Milla, M.E. *et al.* (1999) Specific sequence elements are required for the expression of functional tumor necrosis factor-α-converting enzyme (TACE). *J. Biol. Chem.* 274, 30563–30570

102 Vassar, R. *et al.* (1999) β-Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 286, 735–741

103 Yan, R. *et al.* (1999) Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. *Nature* 402, 533–537

104 Sinha, S. *et al.* (1999) Purification and cloning of amyloid precursor protein β-secretase from human brain. *Nature* 402, 537–540