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A Disintegrin and Metalloproteinase-12 (ADAM12): Function, Roles in Disease Progression, and Clinical Implications

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Abstract

Background—A disintegrin and metalloproteinase-12 (ADAM12) is a member of the greater ADAM family of enzymes: these are multifunctional, generally membrane-bound, zinc proteases for which there are forty genes known (21 of these appearing in humans). ADAM12 has been implicated in the pathogenesis of various cancers, liver fibrogenesis, hypertension, and asthma, and its elevation or decrease in human serum has been linked to these and other physiological/pathological conditions.

Scope—In this review, we begin with a brief overview of the ADAM family of enzymes and protein structure. We then discuss the role of ADAM12 in the progression and/or diagnosis of various disease conditions, and we will conclude with an exploration of currently known natural and synthetic inhibitors.

Major Conclusions—ADAM12 has potential to emerge as a successful drug target, although targeting the metalloproteinase domain with any specificity will be difficult to achieve due to structural similarity between the members of the ADAM and MMP family of enzymes. Overall, more research is required to establish ADAM12 being as a highly desirable biomarker and drug target of different diseases, and their selective inhibitors as potential therapeutic agents.

General Significance—Given the appearance of elevated levels of ADAM12 in various diseases, particularly breast cancer, our understanding of this enzyme both as a biomarker and a potential drug target could help make significant inroads into both early diagnosis and treatment of disease.

Keywords

ADAM12; disintegrin and metalloproteinase; structure; function; inhibitors

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1: An Introduction to ADAMs

The ADAMs (A Disintegrin And Metalloproteinase) are a Zn^{2+} dependant, primarily membrane-bound (although there are some secreted forms) family of multidomain, multifunctional proteins which are members of the zinc protease superfamily [1,2]. There are currently forty gene members known for this family; however only 21 of these are thought to function in humans [3]. In general, the structure of these proteins consists of the following structural elements (shown in Figure 1), beginning with the N-terminus [1,4,5]:

1. A **signal peptide**, which marks the protein for the secretory pathway.
2. A **pro-domain**, which helps to correctly fold the protein as well as prevents the enzymatic activity of the metalloproteinase domain (by a cysteine-switch mechanism) until cleavage in the Golgi apparatus by a pro-protein convertase, or by autocatalysis.
3. A **metalloproteinase** domain, which may or may not have proteolytic activity. The presence of the consensus sequence HEXGHXXGXXHD within this domain has been associated with protease activity, and is found in approximately 50% of the ADAM proteins [4,5].
4. A highly conserved **disintegrin** domain, which plays an important role in cell adhesion, and in binding of ADAMs to integrins [4,5].
5. A **cysteine-rich** domain, which plays roles in substrate recognition and cell adhesion.
6. An **EGF-like** domain, whose function is essentially unknown, and is notably absent in both ADAM10 and 17.
7. A **transmembrane region**.
8. A **cytoplasmic tail**, which has been shown to interact with proteins containing the Src homology domain.

Members of the ADAM family have a variety of roles, including fertility [1], proteolytic shedding of receptor ectodomains [1,6] and protein processing [7,8,9], adhesion [2,6], and cell signaling [1,2].

2: ADAM12

2.1: Structure and Function

2.1.1: Structure—ADAM12 is a proteolytic member of the ADAM family that, as a consequence of alternate splicing, has two forms: a membrane-bound form (ADAM12-L), which has all the domains listed in Figure 1, including the transmembrane and cytoplasmic domains; and a secreted form (ADAM12-S), which is lacking only the transmembrane and cytoplasmic domains [10,11,12] (see Figure 2).

While a full three-dimensional structure of ADAM12 is not yet available, Wewer *et al.* have unraveled the gross structural features of the full length ADAM12-S molecule via electron microscopy. The electron microscopic data suggest that the overall protein molecule has “compact clover shaped architecture and it is composed of four globular domains, one of which is the prodomain.” [13]. The pro-domain appears to remain associated with the metalloproteinase domain, and it has been suggested that it may have some role in the biological activity and/or function of ADAM12-S. Their description of ADAM12-S as visualized by electron microscopy is summarized in Figure 3. Unfortunately, the X-ray crystal structures of ADAM12 are currently unavailable. However considerable sequence

alignment has been reported between ADAM12 and ADAM17 (a.k.a. TACE), and this has served as the basis of comprehending the structural features of ADAM12. Thus in the interest of furthering our discussion, the structure of ADAM17 may be used to provide visual structural elements where such information on ADAM12 is unavailable.

2.1.2: Function and Tissue Distribution—ADAM12 expression is seen most prominently in tissues which are characterized by cell fusion, or growth and/or repair [11,14], such as cartilage [15], bone [15,16], muscle tissue [10,17], adipose tissue [18], liver [19], uterine [20], and brain tissues [21]. The roles played by ADAM12 in these tissues are primarily in cell adhesion and fusion, extracellular matrix restructuring, and cell signaling. While some authors have established the roles of each isoform in healthy adult tissues, the preferential expression of either the –L or the –S form of ADAM12 in healthy human tissue is often ambiguous (see Table 1). *NA = information on role or isoform is not clear from available literature. Overexpression of either or both forms of ADAM12 during the formation and progression of cancers and other diseases has been more clearly documented. An excellent summary of diseased tissue-specific ADAM12-L and –S upregulation has been published by Jacobsen and Wewer [22].

ADAM12 is reportedly involved in the C2C12 myoblast fusion [10], and thus may have important roles in myogenesis [10,11,12]. This binding to C2C12 myoblasts proceeds via the disintegrin-like and cysteine-rich domains of ADAM12. Although they share structural homology with the P-III class of snake venom metalloproteases (SVMPs) [23], they lack the commonly associated RGD integrin-binding motif responsible for binding to structures in the vasculature [24]. Iba et al., further observed that ADAM12 binds to primary murine osteoblasts, fibroblastic cells, osteoblastic cells, and myoblastic cells during the well plate assay in which these cells were seeded to the pre-attached rADAM12 [25]. They report that each of these cells was attached to rADAM12 containing plates and over 90% of them were flattened and spread [26]. In addition, given that the metalloprotease domain of ADAM12 is known to process various extracellular matrix proteins, and that it functions as a gelatinase [27], its role tissue growth and remodeling is easily comprehensible.

2.2: Catalytic Activity

The catalytic structural features of ADAMs, like those of matrix metalloproteinases (MMPs) and other proteases, are described using the nomenclature of Schechter and Berger [28]. The active site of the enzyme is often divided into “subsites” which may recognize additional amino acid residues either local or distal to the scissile bond. Amino acid residues on the peptide substrate recognized by the active subsites of the enzyme are labeled as P1, P2, etc., and P1' P2', etc. to indicate their location in either N- or C-terminal direction relative to the scissile peptide bond, respectively. The enzyme active subsites which recognize these residues are thus labeled S1, S2, etc., or S1', S2' etc. Jacobsen *et al.*, have studied the substrate specificity and catalytic properties of ADAM12-S. They have reported considerable promiscuity of the S1' active site pocket of the enzyme as it accepts Ala, Asp, carboxymethyl (Cm)-Cys (similar to glutamic acid), Leu, Met, Ser, Tyr, and Val residues. To help us visualize these enzymatic pockets, Figure 4 depicts the crystal structure of ADAM17 (which has considerable structural similarity to ADAM12) in complex with hydantoin inhibitor bound in the S1' pocket. In addition to these kinetic features, Jacobsen *et al.* rationalized some of structural-functional aspects of ADAM12 catalyzed reaction. Based on the sequence alignment results, they noted that the enzyme contains a calcium binding consensus motif, which likely plays an important role in the structural stability of the enzyme's catalytic domain [29,30,31,32,33].

An extensive discussion on the mechanistic action of ADAM17 has been presented by Sagi and Milla [34]. We hypothesize that given the considerable structural similarity between ADAM12 and ADAM17, the catalytic mechanism of ADAM12 is likely very similar to this, and therefore we briefly discuss Sagi and Milla's findings. The overall mechanism has been ascribed to involve deprotonation of the active site Zn^{2+} -coordinated water molecule by Glu406 (Figure 5, step 1), followed by the nucleophilic attack of $\text{Zn}^{2+}\text{-OH}^-$ on the carbonyl carbon of the scissile bond by the substrate (Figure 5, step 2). This step results in the formation of an oxyanion intermediate, which is also coordinated by the Zn^{2+} ion, while the amide group abstracts a proton from Glu406 (Figure 5, step 3). Finally, the rearrangement of electrons results in the cleavage of peptide bond cleavage with concomitant regeneration of the enzyme (Figure 5, steps 4 and 5).

2.3: Regulation of ADAM12

ADAM12 regulation is complex, and appears to proceed both during the pre- and post-translational steps. Studies to date have demonstrated a negative regulatory element (NRE) at the 5'-UTR of the human ADAM12 gene which is highly conserved, and it functions as a transcriptional repressor [35]. This stretch of DNA is able to acquire a Z-DNA conformation, which binds to its cognate Z-DNA binding protein hZ α_{ADAR1} [35] (See Figure 6), the binding of which keeps the expression of ADAM12 at a low basal state. It is speculated that reduction in the activity of these Z-DNA proteins plays a vital role in upregulation of ADAM12 expression.

Li *et al.*, have demonstrated that the expression of ADAM12 is also effected by Notch activation of nuclear factor- κB (NF- κB) via a CSL-dependent mechanism [36]. This activation of NF- κB repressed the transcription of microRNA-29 (a suppressor of ADAM12 transcription), which in turn upregulates the transcription of ADAM12. In brief, Notch activates CSL and stimulates the activity of IKK, thereby increasing the proteosomal degradation of NF- κB inhibitors (I κBs), and thus NF- κB is subsequently activated. This activation results in reduced transcription of miR-29, and thus increases the transcription of ADAM12 [36]. A summary of this regulatory pathway is given in Figure 7. The authors further note that only ADAM12-L mRNA contains a target site for miR-29 in its 3'-UTR, and thus it is also susceptible to post-transcriptional regulation by miR-29 (ADAM12-S has no such target site, and thus should not be regulated by miR-29 beyond transcription).

Post-transcriptionally, it has been shown that ADAM12-L interacts with the non-receptor tyrosine kinase, c-Src, causing the redistribution of ADAM12-L to the cell periphery [37]. While under basal conditions ADAM12-L is localized to the peri-nuclear region, activation of c-Src results in tyrosine phosphorylation of the cytoplasmic domain of ADAM12-L, leading to its redistribution to the cell periphery [35] (See Figure 8). During this relocation, the cell phenotype changes to is the highly motile form presumably due to re-organization of the actin cytoskeleton. These features imply that ADAM12-L promotes cellular migration, and given that c-Src is identified as an oncogene in mice, highlights the importance of ADAM12-L in cancer. Beyond the action of c-Src, ADAM12-L has ten possible phosphorylation sites on its cytoplasmic domain, and the resultant phosphorylated forms of the enzyme have been found to be associated with a number of other proteins, such as Abl, and Grb2 [2]. Conversely, Stautz, *et al.* have recently shown that cell surface ADAM12-L may be re-internalized through a clathrin-dependent pathway [38]. Clearly, there are many potential effectors which could modulate the cellular location and activity of ADAM12-L (as reviewed Seals and Courtneidge [2]).

2.4: Involvement of ADAM12 in Disease-Related Ligand Activation and Cell Signaling Pathways

It has been shown that ADAM12 expression may implicate identifiable pathways in disease pathology and they are summarized in sections 2.4.1 and 2.4.2.

2.4.1: ADAM12 Induces EGFR Stimulation via Regulating the Ligand mediated Activation—Growth factor receptor tyrosine kinases (GF RTKs) are a closely related receptor family of proteins, capable of mediating intracellular pathways that are often disrupted in disease. GF RTKs share structural characteristics that allow them to bind common ligands known as epidermal growth factor (EGF) like proteins. Irregularities within RTK-mediated pathways may occur as a result of excess ligand, altered receptor expression, or changes in intracellular signaling, and can lead to a broad array of clinical complications. ADAM12 maintains various roles in the RTK signaling pathway irregularities, which are manifested in different clinical outcomes [39,40].

The prototypical GF RTK is the epidermal growth factor receptor (EGFR). When ligand binds the extracellular domain of the EGFR, it induces the formation of homo- and/or heterodimers, resulting in the phosphorylation of tyrosine residues within the intracellular domain of the receptor. The latter initiates the cascade of signal propagation along specific signaling pathways [41]. As with other GF RTKs, EGFR plays an important role in regulating cell differentiation and proliferation, but has also been linked to the pathogenic processes such as the promotion of various tumors originating in epidermal tissue [42].

Ligands associated with the activation of EGFR are synthesized as transmembrane precursors [42]. The membrane-bound form of EGF-like ligands maintains limited biological activity. But to become fully functional, it must be cleaved in a process known as ectodomain shedding [43]. ADAM12 plays an important role in the regulation of ectodomain shedding, resulting in production of soluble growth factors [39]. HB-EGF is a known EGFR ligand and it serves as an ADAM12 substrate, and thus has been implicated in EGFR dysregulation [39].

A reduction in HB-EGF-mediated EGFR stimulation also was shown to blunt the effects of the vasoactive molecules such as phenylephrine (PE), angiotensin II (AG II), and endothelin-I (ET-1) [44]. Each of these molecules has been shown to have integral roles in heart failure, hypertension, and acute coronary syndromes by inducing physiological changes ranging from vasoconstriction and tachycardia to cardiac hypertrophy and remodeling. The pathways involved in these changes are known to be mediated via the G-protein coupled receptors (GPCRs). Transactivation of EGFR with GPCRs has been shown to enhance compensatory mechanisms of heart failure and ultimately leads to worsening symptoms and/or decompensation [44]. This finding lends support to the idea that ADAM12-mediated HB-EGF cleavage may worsen cardiac remodeling by enhancing the effects of vasoactive GPCR agonists through transactivation of EGFR.

2.4.2: ADAM12 Induces overexpression of HER2/neu Through a Positive Feedback Loop—Her2/neu is another member of the GF RTK family. Although Her2/neu does not have a natural ligand, it is able to heterodimerize with other ligand-bound GF RTK family members [45]. Its presence in various cancers, particularly in breast cancer, has become a prognostic marker and guide for adjuvant treatment; the receptor itself is an important drug target for tumors overexpressing the Her2/neu protein [46]. Pathways downstream of Her2/neu have been shown to promote unchecked proliferation and are commonly mutated in breast cancers [46].

ADAM12 plays an important role in overexpression of Her2/neu protein on the surface of malignant cells. Rao et. al. found that ADAM12 can increase Her2/neu cell surface density via an Ets-1 dependant mechanism [47]. Furthermore, inhibition of Her2 or EGFR stimulation results in the reduction of ADAM12 transcripts, while Her2 transfection elevated ADAM12 expression [47]. This positive feedback loop may help explain what triggers and/or maintains the development of Her2/neu associated tumors.

ADAM12-induced upregulation of Her2/neu surface receptors may be accompanied by a significant increase in EGFR/Her2 heterodimerization. Two primary reasons for this change in cell signaling have been identified: (1) Basal Her2/neu receptor density is barely adequate to allow for efficient heterodimerization with EGFR [48], and (2) EGFR ligand is activated by ADAM12. Although the clinical significance of homo-to-hetero dimerization ratio is not fully understood, the increase in Her2 receptor density may pose several possible complications including enhanced pro-proliferation signal amplitude and/or interference with other pathways directed at balancing the Her2-mediated intracellular activities [48].

Current therapies are available to reduce Her2/neu activity and receptor expression. Trastuzumab, a humanized monoclonal antibody, binds to Her2/neu receptors that are overexpressed on the surface of malignant cells. Upon binding, trastuzumab is able to slow cell growth by preventing dimerization with other GF RTKs, and thus accelerating cellular internalization of the Her2 receptor and promoting cytotoxic T lymphocyte recognition and destruction of affected cells [49]. Although it has shown to be beneficial in certain tumors, this therapy has a wide array of side effects including significant cardiomyopathy [50]. More selective therapies targeting the Her2 pathway and amending the direct causes of Her2 overexpression, via upregulated ADAM12 activity or otherwise, may improve the prognostic outcomes of patients while also reducing the adverse events.

2.5: The Role of ADAM12 in Diagnosis and Progression of Various Disease States

The diagnostic and prognostic value of ADAM12 has been established in a variety of disease states, and for some conditions, ADAM12 levels have been quantitatively correlated with disease progression and outlook. These include various cancers [27,51,52,53,54,55,56,57,58,59], liver fibrogenesis [60], cardiac hypertrophy [61], and asthma [62]. There is also evidence to suggest that increases in ADAM12 are associated with adverse outcomes in pregnancy [63,64,65], and that it may also be used as a first [66] or second [67,68] trimester screen for Down syndrome, although these data are considerably more debated. Given these, it is suggested that discerning the molecular basis of the ADAM12 assisted disease progression would lead to improved selection of therapeutic options and possibly individualized therapy of patients.

2.5.1: Breast Cancer—The most extensively documented disease identified with ADAM12 as a diagnostic and prognostic biomarker is breast cancer, and much is already known about the contribution of ADAM12 overexpression to breast cancer pathology. The presence of ADAM12-S has been documented in the urine of breast cancer patients, and has been well correlated with both the presence of breast cancer and its stage [27]. Roy et al., has demonstrated that as patients progress in the stage of breast cancer (Control (no cancer) < atypical ductal hyperplasia/lobular carcinoma *in situ* < ductal carcinoma *in situ* < inflammatory breast cancer < metastatic breast cancer), the expression of ADAM12-S present in their urine increases progressively against age matched controls (respective means in densitometric units are 12.4 < 23.0 < 47.0 < 68.4 < 85.9) [27]. Figure 9 shows these results. Levels of ADAM12 expression are also correlated with the patient's age at diagnosis, with levels decreasing if the patient was diagnosed at >50 years of age [54].

With these and similar findings in mind, Roy, *et al.* have presented the use of metalloproteinase-cleavable fluorescent resonance energy transfer (FRET) substrates to diagnose breast cancer and determine cancer stage using samples of patients' urine [69]. Using two FRET substrates, named Flsub21 (cleaved by ADAMs -8, -9, -10, -12, and -17; and by MMPs -1, -2, -3, -8, -9, and -13) and Flsub13 (cleaved by ADAMs -8, -9, -10, and -12; and by MMP-2), the authors have screened urine from 85 individuals, including 64 patients at various stages of cancer (ductal carcinoma in situ—24 patients, invasive breast cancer—22 patients, and metastatic breast cancer—18 patients), and 21 age-matched controls. Patient urine samples were incubated with each substrate in triplicate, and any cleavage activity of each substrate was noted. Based on their results, detectable cleavage of both substrates positively identified invasive or metastatic breast cancer with 90% certainty [69]. It must be noted however that the authors were unable to detect ductal carcinoma in situ using this method [69], and thus the use of these polymers as a screen for early breast cancer detection requires further optimization.

Experiments with ADAM12 knockout PyMT mice demonstrate that these knockouts develop tumors at a much slower rate than their ADAM12 expressing controls, and their tumors had reduced growth capacity and fewer lung metastases [55]. Interestingly, it is further shown in this model that ADAM12 from tumor-associated stromal tissues is not necessary for tumor growth, while ADAM12 produced by the tumor itself is required for tumor progression [55]. Of some question is the requirement of proteolytic activity for tumor growth: Frohlich *et al.*, report that proteolytic activity is unnecessary for promotion of tumor growth [55], while Roy *et al.*, report the opposite [52]. It must be noted, however, that the study conducted by Roy was conducted on human samples, while that of Frohlich was conducted on PyMT mice. In addition, Roy notes that only the ADAM12-S resulted in enhanced tumor growth *in vitro*, and *in vivo* caused an increase in metastasis [52], and has further shown in 2012 that overexpression of ADAM12 will promote estrogen independence of breast cancer cell lines [51].

Roy *et al.* demonstrated the effects of ADAM12 on estrogen independence by examining two cell lines (one that overexpressed ADAM12 and one that did not) to determine the role of ADAM12 in ER-positive tumor progression. These authors observed that the proliferation rates were comparable between the two cell lines in the presence of estrogen, but in its absence, cells overexpressing ADAM12 appeared to proliferate faster [51]. However, the estrogen receptor protein levels remained unaffected. In accordance with these findings, subsequent tests found that ADAM12 expressing cells proliferate at a significantly faster rate than the control cells in the presence of tamoxifen, a commonly used antiestrogen therapeutics [51]. The cells that did not express ADAM12 exhibited >50% growth inhibition in the presence of tamoxifen due to a reduction in ER activation [51].

Both ADAM12-L and ADAM12-S acts through a unique mechanism to promote estrogen independence, and thus endocrine therapy resistance. ADAM12-L likely supports estrogen independence through enhanced EGFR expression and MAPK activation whereas ADAM12-S may cause estrogen independence through increased IGF-1R signaling [51]. Upregulating these pathways may preserve pro-proliferative signaling pathway in the absence of estrogen, leading to persistence in tumor growth. Basal expression of each ADAM isoform was comparable in tamoxifen-resistant and tamoxifen-sensitive breast tumor cells [51]. After administration of tamoxifen, however, ADAM12-L and ADAM12-S levels rose sharply, indicating this may be the mechanism by which ER-positive breast cancer cells attain resistance to endocrine therapies [51].

2.5.2: Bladder Cancer—Findings from bladder cancer studies essentially mimic those found in breast cancer: levels of ADAM12-S increase in urine as a function of cancer stage

[56]. It has also been reported that these levels drop following removal of the tumor mass, and increase again following tumor recurrence [56].

2.5.3: Lung Adenocarcinoma—There is evidence that overexpression of ADAM12 is responsible for an increase in cell proliferation and invasion in stably transfected human bronchial epithelial cells [58], and it correlates well with poor patient prognosis [59]. Rocks *et al.*, transfected human bronchial epithelial cells (BEAS-2B) with pcDNA3-*neo* plasmid containing the full length human ADAM12-L gene, causing these cells to overexpress the membrane-bound form of ADAM12 [58] (ADAM12-S was not included in this study, as the authors have previously reported that ADAM12-S is not overexpressed in either lung tissues or tumors [57]). When tested *in vitro*, these cells demonstrated increased proliferation, and reduced apoptosis; this is attributed to increased shedding of the heparin-binding epidermal growth factor-like growth factor (HB-EGF) from the cell surface. Further supporting this view, antibodies against HB-EGF stunted this proliferation when added to the culture media, and reduced the protection against apoptosis (as induced by etoposide) demonstrated by these cells in the absence of antibody [58].

Mino *et al.*, have investigated the effects of high vs. low expression of ADAM12-L in patients with stage-I lung adenocarcinoma [59]. Prior to these studies they have quantified the ADAM12-L and -S expression in 19 human lung cell lines (both cancerous and non-cancerous) and have found (as did Rocks *et al.*) that there is little or no ADAM12-S expression in these cells [59]. As such, they too have focused only on expression of ADAM12-L. They report that the group of patients expressing high levels of ADAM12-L had less differentiated tumors, a higher recurrence of cancer following treatment, and a lower overall 5-year survival rate than those expressing lower levels of ADAM12-L [59].

2.5.4 Brain Tumors—Kanakakis, *et al.* have recently established the presence of ADAM12 in healthy human oligodendrocytes [21], as well as in low grade oligodendroglial and astrocytic neoplasms. Interestingly, these authors report an inverse correlation between ADAM12 and tumor malignancy, with ADAM12 mRNA decreasing as tumor malignancy increases [70]—e.g. low grade oligodendrogliomas (WHO grade II) stained positive for ADAM12 at the same frequency as healthy brain tissue, with approximately 70% of cells expressing protein. Anaplastic oligodendrogliomas (WHO grade III) expressed the ADAM12 protein in only 40–50% of cells. In addition, quantities of ADAM12 mRNA were 18-fold higher in low grade tumors compared with high-grade tumors [70]. These authors also report that overexpression of ADAM12 did not co-localize with glial fibrillary acidic protein (GFAP), an intermediate filament protein expressed by various cell types of the central nervous system, and which is associated with CNS injury and formation of glial scars [71]. This selectivity suggests that ADAM12 may be a useful biomarker for identifying oligodendroglial cell subpopulations in mixed brain tumors [70].

2.5.5: Liver Fibrogenesis—Bourd-Boittin *et al.*, have demonstrated that ADAM12 is a binding partner for the receptor for activated protein kinase C (RACK1), a homolog of the β -subunit of heterotrimeric G proteins [60]. Both RACK1 and ADAM12 are overexpressed in patients with hepatic carcinomas. By treating human hepatic stellate cells from partial hepatectomy patients with PMA (phorbol 12-myristate 13-acetate, used as an activator of PKC [72]), they demonstrate that translocation of ADAM12 to the cell surface is modulated by PKC activity. They further suggest that upstream from this phenomenon, type I collagen (a hallmark of the injured liver [73]) interacts with β -integrin, activating PKC, and allowing the translocation of ADAM12 to the cell surface via a RACK1-dependent pathway [60]. Once at the cell surface, ADAM12 may contribute to cell spreading and cancer progression.

2.5.6: Cardiac Hypertrophy and Hypertension—Wang *et al.*, report a transcriptional and posttranscriptional link between MMP7 and ADAM12 which appears to contribute to hypertension in mouse and rat models [61]. Upon treatment of already hypertensive SHR mice at 22 weeks of age with MMP7 siRNA, the authors noticed a decrease in systolic blood pressure, and reduced progress of cardiac hypertrophy. However, they further noticed that administration of MMP7 siRNA also inhibited transcription of ADAM12. While continuous infusion of angiotensin II caused hypertension and hypertrophy of the left ventricle in mice, a decrease in MMP7 transcription and corresponding increase in ADAM12 transcription were observed. Treatment of these mice with MMP7 siRNA prior to angiotensin II infusion resulted in reduced transcription of ADAM12, and resistance to hypertension and cardiac hypertrophy [61].

Asakura *et al.* demonstrated that cardiac hypertrophy, a hallmark sign of heart failure, could be inhibited by antagonizing the proHB-EGF sheddase activity of ADAM12 and thus limiting the availability of soluble HB-EGF [44]. The observed reduction in cardiac growth is likely to be the result of diminished EGFR stimulation, secondary to reduced ligand binding. Moreover, Asakura further reported that an inhibitor of ADAM12 (KB-R7785, see Figure 5) reduces cardiac hypertrophy in mice by blocking the enzyme-mediated shedding of HB-EGF [44], further suggesting the importance of ADAM12 in hypertension and cardiac hypertrophy. This study is further discussed in the section on synthetic ADAM12 inhibitors.

2.5.7: Asthma—Estrella *et al.*, have shown that ADAM12 is upregulated in bronchial epithelial tissues in response to proinflammatory stimulation by TNF- α [62]. Further, the ADAM12 expression was shown to be increased in the airway epithelial cells of allergic patients following exposure of these patients to their allergens [62]. This upregulation was further associated with alterations in cell adhesive properties, and the expression of integrins and cytokines. As ADAM12 has also been associated with tissue remodeling and changes in the extracellular matrix (phenomena associated with chronic asthma [74]), it is thought that ADAM12 may contribute to the remodeling of the bronchial airways as well as recruitment and accumulation of neutrophils in the airway mucosa, assisting the formation of chronic asthma symptoms.

2.5.8: Adverse Pregnancy Outcomes and Down Syndrome—It was proposed in 2006 that low levels of ADAM12 in maternal serum are correlated with Down syndrome [66]. These authors reported significantly reduced levels of ADAM12 in the serum of women in their first trimester of pregnancy having infants with Down syndrome, suggesting that ADAM12 levels correlate well with occurrence of Down syndrome, and thus would constitute a good first-trimester screen for the disease. However in contrast to this, Poon *et al.*, reported the opposite in 2009, suggesting that ADAM12 levels in the maternal sera in Down syndrome pregnancies are no different from those of normal [75]. Additionally, Christiansen *et al.*, suggested that while screening ADAM12 levels is a “moderately effective DS marker”, it is not cost effective [76]. Clearly results in the first trimester are highly variable, and as such they are unlikely to constitute a reliable Down syndrome screening regimen.

Despite unreliable results in the first trimester, results in the second trimester have shown more promise. Both Christiansen and Wang have reported good correlation between ADAM12 levels in maternal sera and Down syndrome, with levels of ADAM12 increasing with respect to controls [67,68]. Whether screening ADAM12 levels in maternal sera will finally yield a reliable test for Down syndrome has yet to be determined.

Other adverse pregnancy outcomes have also been suggested to be correlated with low ADAM12 serum levels, such as preeclampsia, spontaneous pre-term delivery, or delivery of neonates that are small for their gestational age [63,64,65,77]. However Poon *et al.*, has reported that ADAM12 is not correlated with these outcomes at all [78], and thus results are again likely too varied to result in reliable predictions.

3: ADAM12 Inhibition

3.1: TIMPs—Natural Inhibitors of ADAM12

As discussed previously, ADAM proteins are likely to be upregulated *in vivo* in response to biological stimuli or pathological conditions intrinsic to the diseased states. The body's innate ability to modulate ADAM activity is maintained by tissue inhibitors of metalloproteinases (TIMPs), a group of proteins originally identified as inhibitors of MMP [79]. The TIMP family consists of four isoforms of protease inhibitors, TIMP1–4, that have broad but varying affinity for specific MMP, ADAM, and ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin Motifs) isozymes [80,81]. TIMP-2 and TIMP-3 exhibit no selectivity in inhibiting ADAM12 activity. Efforts have been made to modify the structures of TIMPs in order to improve specificity and affinity for the ADAM12 [82], and recent work with other ADAMs suggest this potential [83].

TIMP-3 exhibits a broader array of inhibitory potential as compared to other TIMPs, including the inhibitory effects on a variety of ADAM proteins [82]. Loechel *et al.*, found that in equimolar ratios, TIMP-3 inhibited the cleavage of IGFBP-3 and IGFBP-5 by 32%, in a 2:1 ratio it inhibited ADAM12 by 51%. At a 10:1 ratio of TIMP-3 to ADAM12, the inhibition was nearly 100% [84]. The apparent inhibition constant ($K_{i(app)}$) of N-TIMP-3 toward ADAM12-S protein was measured to be 12.5 ± 1.1 nM, much greater than that of TIMP-1 and slightly better than TIMP-2 [29]. The relative potency of TIMP-3 indicates that it may be the primary physiological regulator of ADAM12 activity. Despite its efficacy, the general lack of specificity may limit direct clinical applications of TIMP-3, as it maintains activity against every physiological MMPs and several ADAMs [82].

TIMP-2 also inhibits ADAM12 activity, but to a lesser extent than TIMP-3 [29,85]. As observed with TIMP-3, structural modification in TIMP-2 has been shown to improve its inhibitory potency for ADAM12. With comparable efficacy, the relative selectivity of TIMP-2 may confer a potential advantage over TIMP-3 [82]. Kveiborg and colleagues have found that the N-terminal domain of TIMP-2 has improved activity toward ADAM12, with apparent binding affinity (i.e., inhibition constant, K_i) increasing from 84 ± 13.6 nM for TIMP-2 to 69.7 ± 8.8 nM for N-TIMP-2 (each for the full ADAM12-S sequence) [85]. Upon removal of the AB-loop from TIMP-2, the apparent binding affinity increased to 28.1 ± 2.4 nM [78]. These authors report the binding of the N-terminus of TIMP-3 at 4.4 nM for ADAM12-S, while Jacobsen reports its magnitude to be 12.5 ± 1.1 nM.

3.2: Synthetic ADAM12 Inhibitors

A number of synthetic molecules targeting the catalytic domain of ADAM12 have been reported. One such inhibitor is KB-R7785, a GM6001-derived hydroxamate [44] (structure is given in Figure 10). KB-R7785 was originally synthesized by Hirayama *et al.*, at the New Drug Discovery Research Laboratory in Osaka, Japan [86], and is now commercially available from Organon Japan (Osaka, Japan). Since 1997, KB-R7785 has been used in a variety of studies as a matrix metalloproteinase (MMP) inhibitor. Studies have shown that KB-R7785 curtails production of TNF- α (possibly by inhibiting the action of ADAM17) [87], inhibits cell migration by inhibiting ADAM10 processing of CD44 [88], and inhibits migration of osteoclasts while prolonging the life of podosomes by inhibition of MMPs [89]. The inhibition of ADAM12 by KB-R7785 was explored by Asakura *et al.*, wherein intra-

peritoneal injections of KB-R7785 (100 mg/kg/day) were administered to male C57BL/6J mice (8 weeks old) or male FVB mice (7 weeks old), which also received infusions of phenylephrine (PE) and angiotensin II (AngII) to induce cardiac hypertrophy. Controls in this study received PE and AngII, but no KB-R7785 [44]. Results indicated that heart weight and ratio of heart weight to body weight were significantly decreased by KB-R7785, indicating abrogation of cardiac hypertrophy. The heart rate, blood pressure, and body weight were unaffected [44].

Another synthetic ADAM12 inhibitor is TMI-1, a sulfonamide-hydroxamate, first synthesized by Zhang *et al.*, as an inhibitor of ADAM17 and various MMPs as a potential treatment of rheumatoid arthritis [90] (structure is given in Figure 10). These authors report successful inhibition of ADAM17 and other MMPs at nanomolar concentrations, and significant reductions in arthritis clinical severity scores in their mouse model [90]. Furthermore, these authors have given oral doses of drug (50 mg/kg) to Balb/CJ mice, and found the pharmacokinetic properties potentially suitable for oral dosing (results summarized in Table 2). In 2007, Moss and Rasmussen reported TMI-1 strongly inhibited ADAM12 in buffer solution (20 mM Tris, pH 8.0) with a reported binding constant of 1.8 ± 0.1 nM [91]. To date, no studies are published validating the use of TMI-1 for inhibition of ADAM12 *in vivo*.

Finally, Oh *et al.*, describe a dipeptide analogue called “Compound 5” with a reported IC_{50} of 16.7 nM [92] (structure shown in Figure 10). The structure is the product of virtually screening a large library of 67,062 compounds, all having a Zn^{2+} -binding moiety (hydroxamate, carboxyl, or thiol groups). A 3D model of ADAM12 was built using reference enzymes with >30% homology to ADAM12 (acutolysin-C, and adamalysin). Screening of this library eventually yielded 64 compounds for biological testing, for which they have confirmed activity using a cell-based assay. In this assay, they have stably transfected *Drosophila* S2 cells with plasmids encoding for a triple fusion protein: Golgi α -mannosidase II (GMII), which is anchored to the Golgi membrane; and alkaline phosphatase (AP) connected by an ADAM12 substrate sequence [92,93]. As ADAM12 cleaves its consensus sequence, AP is released into the cell culture media, and detected by colorimetric measurement [92]. Based on results from this assay, the group has determined the relative IC_{50} 's of four of their screened compounds showing the most promise, with Compound 5 having the greatest inhibition [92]. Despite the success of these aforementioned molecules in inhibiting the proteolytic activity of ADAM12, targeting of the enzyme as a treatment for disease is still problematic. It is crucial in design of inhibitor drugs to achieve specificity for the enzyme of interest. However, there is a great deal of structural similarity between the active sites of the metalloproteinases [94,95]. It has been suggested that, rather than targeting the catalytic domain of the protein, greater success could be achieved by targeting one of the other domains. Takeda *et al.*, and Igarashi *et al.*, have explored the hypervariable loop region of the cysteine region of ADAMs, and have suggested this as an attractive targeting site for inhibition [96,97]. It has been shown that mesenchymal cells will bind to the cysteine-rich domain of ADAM12 via syndecans, and will spread to form focal adhesions and organize actin fibers following this binding [26]. Carcinoma cells also bind to ADAM12 via syndecans, however they fail to spread [25] (although they may be induced to spread by addition of 1 mM Mg^{2+} , or antibodies targeting $\beta 1$ integrin (mAb 12G10) [26]). Drugs targeted to the cysteine-rich region of ADAM12 have potential, therefore, to inhibit adhesion and invasion of metastases, however more research is required to develop such a drug, and confirm its efficacy.

4: Conclusion

ADAM12 is a member of the Zn²⁺ dependent metalloproteinase superfamily, and is expressed as both a membrane-bound and a secreted enzyme. Overexpression of ADAM12 is implicated in the onset and progression of a number of disease conditions, including various cancers, liver fibrogenesis, cardiac hypertrophy, hypertension and asthma, and changes in its maternal blood serum levels are associated with second-trimester Down syndrome. ADAM12 has potential to emerge as a successful drug target, although targeting the metalloproteinase domain with any specificity will be difficult to achieve due to structural similarity between the members of the ADAM and MMP family of enzymes. Overall, more research is required to establish ADAM12 being as a highly desirable biomarker and drug target of different diseases, and their selective inhibitors as potential therapeutic agents.

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Highlights

- ADAM12 has been implicated in cancers, liver fibrogenesis, hypertension, and asthma.
- We provide an overview of the ADAM proteins and their roles in normal physiology.
- We discuss the role of ADAM12 in the progression and diagnosis of various diseases.
- We discuss currently known natural and synthetic inhibitors.
- We conclude that ADAM12 has the potential to emerge as a successful drug target.

Figure 1.
General structure of ADAM proteins

Figure 2.
Structures of ADAM12-L and -S forms

Figure 3. Schematic illustration of the structure of ADAM12-S, as visualized by electron microscopy [13].

Figure 4. Catalytic domain of ADAM17 in complex with hydantoin inhibitor in the S1' pocket, rendered using Chimera software (Version 1.5.3); (A) ribbon structure showing Zn²⁺ (magenta) coordinated by three histidine residues; (B) hydrophobic surface depicting hydrophilic residues in blue, hydrophobic residues in red. Shown are the hydantoin inhibitor (green) and Zn²⁺ ion (magenta). (C) two-dimensional structure of hydantoin inhibitor. PDB ID# 3L0V.

Figure 5.
Proposed mechanism of ADAM17 enzymatic cleavage [34].

Figure 6. When Z-DNA binding protein hZα_{ADAR1} is bound to the 5'-UTR, DNA transcriptase cannot bind (A). In tissues where hZα_{ADAR1} is not present, DNA transcription can take place, resulting in the upregulation of ADAM12 (B).

Figure 7.
Regulation of ADAM12-L transcription by Notch signaling [36].

Figure 8.
Post-transcriptional regulation of ADAM12-L by c-src.

Figure 9.

Presence of ADAM12-S in urine of breast cancer patients correlates with cancer status and stage [27]. Respective means in densitometric units are shown. Graph was generated from data presented in the original manuscript.

Figure 10.
Structures of reported ADAM12 inhibitors.

Table 1

Role of ADAM12 in normal healthy tissues

Tissue	Reference	Role	Isoform
Cartilage	[15]	<ul style="list-style-type: none"> • Modulate chondrocyte proliferation 	S
Bone	[15,16]	<ul style="list-style-type: none"> • Bone elongation through proteolysis and mediation of cell-matrix interactions • Formation of osteoclasts from peripheral blood monocytes 	S
Muscle	[10,17]	<ul style="list-style-type: none"> • Formation of reserve cell pool through cell-cell interactions • Induces quiescence during myogenic differentiation 	NA*
Adipose	[18]	<ul style="list-style-type: none"> • Actin cytoskeleton reorganization • Reduced cell adhesion • Impaired $\beta 1$ integrin function 	NA
Liver	[19]	<ul style="list-style-type: none"> • Extracellular matrix remodelling during fibrogenesis 	NA
Uterus	[20]	<ul style="list-style-type: none"> • Decidualization during early stages of pregnancy 	NA
Brain	[21]	NA	

Table 2

Pharmacokinetic properties of TMI-1 [90]

C _{max}	3 μ M
T _{1/2}	1.68 hr.
AUC	1484 ng/hr/mL
Bioavailability	39%