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Therapeutic Benefits from Targeting of ADAM Family Members†

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ABSTRACT: Members of the ADAM (a disintegrin and metalloproteinase) family of proteolytic enzymes are implicated in the processing of many single transmembrane-bound proteins ranging from cell surface receptors to growth factors and cytokines. Because of the biological significance of these processing events, a recurring theme in studying ADAM biology is that they are involved in physiological processes that can go awry and lead to disease states. This review provides a comprehensive look at ADAM family members and their role in pathology and provides a pathway for determining whether an enzyme is a physiological convertase for a given protein. In addition, ADAMs are discussed as potential therapeutic targets.

Single-span transmembrane proteins contain an anchor or transmembrane segment with either their N- or C-terminus in the extracellular space. Regardless of how these membrane proteins are placed in the membrane, processing by proteolytic enzymes occurs, in many cases, to release soluble forms with important biological functions (reviewed in ref *1*). While cleavage of membrane proteins may occur within the cell, for example, in the Golgi or in the plasma membrane, both events have been termed ectodomain shedding and are typically carried out by members of the ADAM[1] (a disintegrin and metalloproteinase) family (*2*). ADAM family members typically contain a pro domain that keeps the enzyme in an inactive state. Pro domain removal occurs either through the action of pro-hormone convertases or by autocatalysis. In addition, ADAM proteins contain catalytic and disintegrin domains, a transmembrane region, and a cytoplasmic tail (Figure 1). Currently, there are 39 reported sequences of ADAMs, and researchers are just beginning to unravel their importance in terms of physiological roles and as pharmaceutical targets.

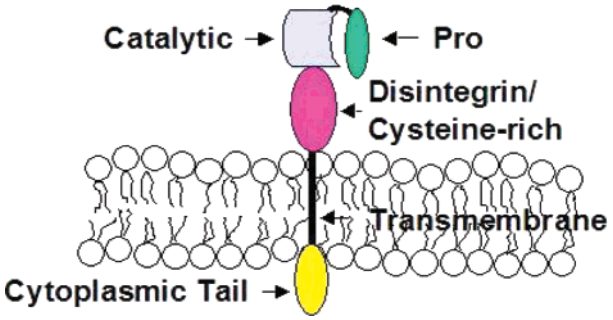

FIGURE 1: Domain structure of ADAM family members. ADAM proteases contain a pro domain, a catalytic or metalloproteinase domain, a disintegrin and cysteine rich region, and a transmembrane segment (in black), along with a cytoplasmic tail. Upon removal of the pro domain, either by furin proteases (e.g., PC3) or by autocatalysis, the metalloproteinase domain is activated.

The metalloproteinase activity for ADAMs has been the main focus of researchers in general, with reports on substrate specificity and inhibitor studies accumulating in the literature.

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For example, ADAMs possess broad substrate specificities as shedding of growth factors, cytokines, cell adhesion molecules, and receptors is often carried out by these proteins. ADAMs are currently implicated in the processing of precursor forms of proteins such as TNF-α (*3*, *4*), TGF-α (*5*, *6*), HB-EGF (*7*−*10*), amphiregulin (*7*, *11*), APP (*12*, *13*), Notch (*14*, *15*), IL6R (*16*), IL1-RII (*17*), CD163 (*18*), GHBP (*19*), L-selectin (*5*), the neural recognition molecule L1 (*20*), the close homologue of L1, CHL1 (*21*), TNFRI (*16*, *17*), TNFRII (*5*, *22*), the M-CSF receptor (*23*), the low-affinity receptor for IgE, CD23 (*24*), the erbB4 receptor (*25*), and type 1 neuregulin (*26*). For some of the substrates mentioned above, conflicting or insufficient data for determining the physiological convertase(s) have been reported.

Physiological Convertases: TACE and ADAM 10

While studies performed with cells from TACE-deficient mice suggest that this ADAM is an actual convertase for a number of shedding events, many parameters can be assessed in identifying a physiological processing enzyme. The most direct and simplest experiment is to use peptides spanning the cleavage site of a putative substrate. Cleavage reactions *in vitro* can be performed to determine, first, if cleavage occurs and, second, if cleavage occurs at the physiological site. Peptide cleavage reactions should not be the only criterion used when defining a physiological convertase, as often ADAM family members can cleave peptide substrates *in vitro*, but are not actual convertases for a given substrate. Next, *in vitro* reactions can be performed using full-length substrates. Again, identification of cleavage sites is critical for ascertaining whether an ADAM is acting as a physiological convertase. Third, cell-based assays can be implemented to carry out cotransfection experiments such as introducing siRNAs, or dominant negative constructs (these constructs are typically mutant convertases that act by sequestering factors away from the wild-type enzyme which are necessary for catalytic function). Cell-based assays can also be performed with inhibitor studies in which a known convertase inhibitor is tested for its ability to inhibit release of the mature forms of factors. Inhibitor studies may also be difficult to evaluate if multiple enzymes process a factor and if only a particular cell type is studied. Finally, *in vivo* knockout studies should be performed both to examine the overt phenotype of the mice and to determine deficiencies in processing events.

In fact, all of the criteria mentioned above have been met in ascertaining whether TACE/ADAM 17 is an actual TNF-α-converting enzyme. TACE was purified on the basis of its ability to cleave both peptide and full-length protein TNF-α-based substrates at their physiological sites (*3*, *4*). Inhibitors of TACE also proved to be effective in cell-based assays in preventing release of soluble cytokines (*27*). In addition, knockout animals were generated, and immunocompetent cells from these animals also proved to be deficient in the release of soluble TNF-α (*3*). All of these factors, taken together, provide substantial proof that TACE is a TNF-α-converting enzyme.

One example, in which only some of the criteria were used to provide proof for a physiological convertase, led to the identification of ADAM 10 as a TNF-α-converting enzyme. For example, ADAM 10 is also able to process the same TNF-based substrates as TACE, but with reduced efficiency. However, reports based on purification protocols by two groups suggested that ADAM 10 was a physiological TNF-α-converting enzyme (*28*, *29*). Cell-based assays using inhibitors of ADAM 10 have, however, ruled out this enzyme as an actual TACE, providing support for the notion that multiple criteria are needed to define what constitutes a physiological convertase. Thus, a continuing and perplexing problem in the study of ADAM family members is to ascertain whether they are physiological convertases, and also if there are in fact multiple convertases for a variety of substrates as some of the studies suggest.

Processing of Growth Factors

Experiments with cells from TACE and ADAM 10 deficient (mutated and therefore nonfunctional catalytic domain) mice as well as their overt phenotype have provided insights into what substrates are physiologically relevant. TACE is most likely a processing enzyme for TGF-α since the TACE null mice exhibit open eyes and wavy hair at birth and this phenotype is comparable to what is found for TGF-α deficient mice (*5*, *30*, *31*). TGF-α is processed at two sites: N- and C-terminus. It is the C-terminal cleavage reaction that leads to release of membrane-bound TGF-α. Convincing evidence points to TACE as a physiological N- and C-terminal convertase (*7*). Other groups, however, have discovered TGF-α processing activities separate from TACE, indicating that processing of this growth factor is quite complex. For example, N-terminal processing can also be carried out by ADAM 10, albeit less efficiently (*6*). In addition, a novel membrane C-terminal convertase activity separate from TACE was discovered in R1 epithelial cells (*6*). TACE is also implicated in the shedding of other factors in the EGF ligand family such as HB-EGF and amphiregulin as the phenotype of the mutant mice exhibits an EGF receptor knockout phenotype (*7*, *32*−*34*). For example, TACE knockout mice exhibit the same heart defects that occur in HB-EGF knockout mice (*35*, *36*). Also, in cotransfection experiments with immortalized fibroblasts from mutant TACE mice, TACE can process both amphiregulin and HB-EGF, suggesting that TACE is an actual convertase for these substrates, and while cleavages can occur at multiple places that include the physiological sites (*7*), the cumulative data support the fact that it is an *in vivo* convertase for the EGF ligand substrates described above.

In addition, studies performed with *in vitro* assays provide support for ADAM 10 involvement in EGF ligand processing. For example, ADAM 10 is considered an HB-EGF processing enzyme since it is required for G-protein-coupled activation of this EGF ligand family member (*10*). However, *in vitro* processing of HB-EGF peptide substrates by ADAM 10 does not occur even though it can process the N- and C-terminal peptide and partial-length substrates based on TGF-α (*6*, *36*). While the N-terminal TGF-α cleavage reactions occur at the physiological site, C-terminal cleavage does not, suggesting that ADAM 10 is an N-terminal but

[1] Abbreviations: ADAM, a disintegrin and metalloproteinase; APP, amyloid precursor protein; CHL1, close homologue of L1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; GHBP, growth hormone binding protein; HB-EGF, heparin-binding epidermal growth factor; IL6R, interleukin 6 receptor; IL1-RII, interleukin 1 receptor type II; M-CSF, macrophage colony-stimulating factor; MADM, mammalian and disintegrin metalloproteinase; TACE, TNF-α-converting enzyme; TGF-α, transforming growth factor-α; TNFRI, tumor necrosis factor receptor I; TNFRII, tumor necrosis factor receptor II; TRANCE, TNF-related activation-induced cytokine.

not C-terminal convertase. Support for N-terminal convertases other than TACE comes from previous experiments with mutant cells that are deficient in multiple metalloproteinase-dependent shedding events (*37*). In these cells, TACE cannot efficiently exit the endoplasmic reticulum. Therefore, its pro domain is still attached, keeping the enzyme in an inactive state (*38*). N-Terminal processing of TGF-α can occur, while C-terminal cleavage and release of mature TGF-α cannot take place in these cells. A reasonable proposal is that ADAM 10 is acting as an N-terminal convertase in mutant cells.

Processing in the Nervous System: APP and Notch

Like EGF ligand release, studies with TACE and ADAM 10 involving APP and Notch processing are also complex. APP is cleaved at three positions, and one site termed the "α site" is thought to be processed *in vivo* by ADAM family members ADAM 17 and/or ADAM 10 (reviewed in ref *39*). In normal APP secretion, cleavage at the α and γ sites occurs to generate fragments that are important for brain function (normal amyloid deposition). However, in Alzheimer's disease, β-amyloid plaque formation occurs through processing of APP at the β site by an aspartyl protease named BACE and γ sites via a presenilin-dependent process (reviewed in ref *40*).

Biochemical experiments *in vitro* support TACE and ADAM 10 as α secretases. Both can, for example, process a peptide substrate based on the α cleavage site (*12*, *13*). Furthermore, transfection experiments with a dominant negative form of nonfunctional ADAM 10 protein provide evidence that it may be a physiological α secretase (*13*). Yet for APP processing, 15 of 17 immortalized fibroblast cell lines generated from ADAM 10 deficient mice were not deficient in constitutive and PMA-stimulated APP cleavage, ruling out ADAM 10 as major α secretase (*15*). A caveat is that since the embryos die so young before the nervous systems are developed, fibroblasts established from the knockout animals are not representative of what occurs in normal physiology. In contrast to the situation in ADAM 10 deficient mice, PMA-stimulated APP processing in immortalized fibroblasts from ADAM 17 null mice is impaired, pointing to a role for TACE in amyloid processing under inflammatory conditions (*12*). An experimental approach using siRNA constructs demonstrated that at least three ADAMs (9, 10, and 17) are involved in physiological APP processing (*41*).

Like APP processing, Notch release requires multiple steps. Notch is a receptor that is important in neuronal development. It is processed at three sites by different proteins via a process that ultimately generates a cytosolic fragment that enters the cell nucleus and interacts with transcription factors, to carry out signaling events. The "site 2" cleavage event can occur *in vitro*, by TACE (*14*). However, genetic evidence from both invertebrate and vertebrate knockout studies points to ADAM 10 as a physiological convertase for Notch processing. For example, knockouts of the orthologues of ADAM 10, Kuzbanian, and SUP 17, in invertebrates, *Drosophila*, and *Caenorhabditis elegans*, respectively, result in deficiencies in Notch signaling such as cardiac and neuronal defects (*42*, *43*). In addition, the phenotype of knockout ADAM 10 mice is consistent with the knockout phenotype for the Notch 1 receptor, as there are defects in neuronal development and embryos die on day 9.5 (*14*). These data overwhelmingly support a role for ADAM 10 in Notch cleavage.

Early experiments with TACE were also supportive of a role for this enzyme in Notch activation (*14*). However, transgenic mice with a mutant and inactive TACE gene do not have a Notch knockout phenotype. This finding provides significant proof that TACE is most likely not a Notch convertase. Therefore, even though peptide cleavage reactions suggest that both ADAM family members are involved, only one metalloproteinase, ADAM 10, is an actual convertase for Notch processing.

Shedding of Membrane Proteins and G-Protein-Coupled Activation

After TACE was purified and cloned and considered an actual tumor necrosis factor α-converting enzyme (*3*, *4*), investigators put more emphasis on studying all facets of disintegrin metalloproteinases, from their pro domains to their cytoplasmic tails. These domains contain motifs with relevance to signaling, e.g., SH3 domains suggesting that they are involved in intracellular signaling. From work pioneered by Ullrich, it was first demonstrated that metalloproteinases were coupled to G-protein activation and ligand release resulted in activation of members of the epidermal growth factor receptor family (*44*). Subsequently, reports implicated disintegrin metalloproteinases as the link between G-protein-coupled responses and EGF receptor activation.

Once G-proteins are activated through agonist binding, ADAM family members are themselves "activated". Plausible mechanisms are pro domain removal or regulation of protease activity by cytoplasmic domains. Another intriguing possibility is the localization of ADAMs and substrates in microdomains of the plasma membrane (lipid rafts). Lipid rafts are localized regions of elevated cholesterol and glycosphingolipid content within the plasma membrane. In some cases, enzymes such ADAM 10 and TACE are sequestered from lipid raft-containing substrates and activators of ADAMs such as the pro-hormone convertase, PC3 (*45*). Shedding by ADAM family members seems to be dependent on the membrane fluidity and the lipid raft composition. At least for ADAM 10 and ADAM 17, experimental evidence which shows that a low level of cholesterol or even cholesterol depletion favors shedding of CD30 (*46*), APP (at the α secretase site; *47*, *48*), and L1 (*49*). In contrast to these studies, ADAM 19-mediated ecto domain shedding is localized to lipid rafts (*50*), suggesting that complex formation of ADAMs with other membrane proteins could be critical in the regulation of ecto domain shedding.

While cholesterol levels seem to affect shedding of cell surface molecules, it is still unclear how ADAM family members become activated, especially under conditions where cholesterol levels remain constant. One plausible mechanism is that lipid rafts also include signaling complexes containing tetraspanins. The tetraspanins are cell surface proteins that span the membrane four times, forming two extracellular loops (reviewed in ref *51*). Tetraspanins form complexes at the cellular surface, possibly with ADAM family members, and integrins. Tetraspanins such as CD81 can prolong the association of certain receptors with signaling active lipid rafts. For example, in tetraspanin CD81 deficient B cells, coligated B cell receptor (BCR) and CD19−CD21

complexes fail to partition into lipid rafts and enhance BCR signaling from rafts.

For the ADAM family members, the tetraspanin CD9 can associate with ADAM 10, a substrate for ADAM 10, HB-EGF, and integrins, $\alpha_3\beta_1$ (*52*) and $\alpha_6\beta_1$ (*53*). While it is not yet clear what role CD9 plays, protein kinase C (PKC) directly associates with this tetraspanin, possibly via the cytoplasmic tails of the first two transmembrane domains. The tetraspanins could act as localization molecules bringing PKC or other activators into the proximity of the ADAMs so that modifications such as phosphorylation of the cytoplasmic tail and/or pro domain removal can be introduced (*53*).

While the literature suggests that tetraspanins bind directly to integrins, a putative TACE/ADAM 10 cleavage site was found in CD9 through computer searches using the Blast web site. When the transforming growth factor-α (TGF-α) processing site for the N-terminus, SPVAAAVVSHFN, was used as a query, amino acids 101−110 (EVAAAVVALV) in the extracellular loop of CD9 were identified as a possible binding or cleavage site. Perhaps, as a regulatory step, ADAMs such as TACE and ADAM 10 are able to process CD9. Interestingly, others have reported that this adjoining region in CD9 (residues 112−154) is an epitope whose expression depends on changes in the activation state of the $\alpha_6\beta_1$ integrin and is only exposed upon CD9 binding (*54*). A hypothetical mechanism for activation involving CD9 and its association with ADAMs and integrins is described in Figure 2. In this hypothetical mechanism, CD9 localized to lipid rafts directly interacts with ADAM 10 through the substrate site (residues 101−110). The complex contacts integrins through the disintegrin domain of the ADAMs. Since the integrins carry out signaling events, in some instances, through GTPases, and are also localized to lipid rafts (*55*), complex formation would link ADAM activation to G-protein-coupled responses.

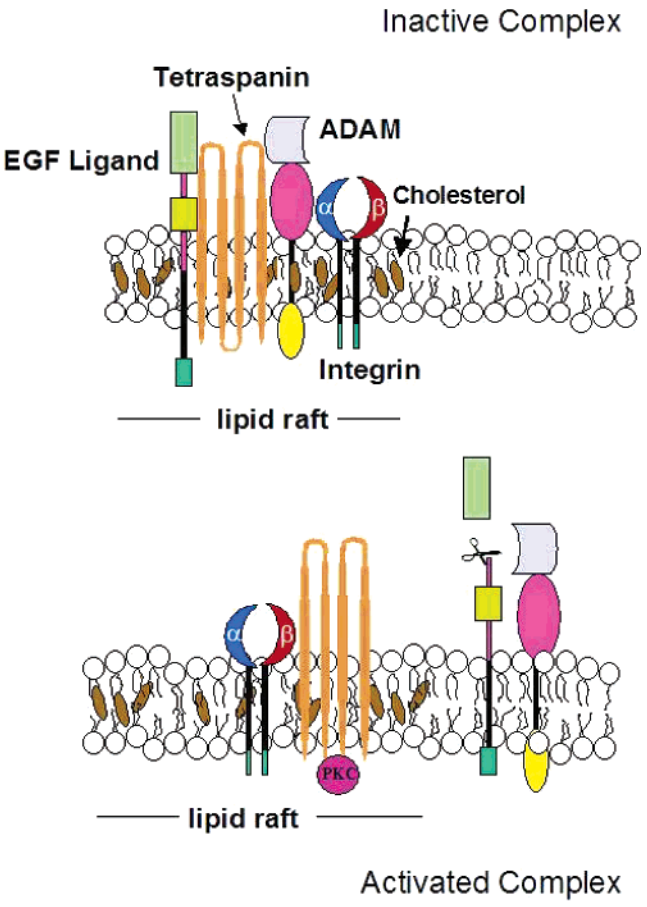

FIGURE 2: Association of tetraspanin (e.g. CD9) with ADAM 10/TACE, integrins, and lipid rafts. ADAM 10 and TACE may be able to interact with one of the extracellular loops of tetraspanin (e.g., CD9) directly to form an inactive complex within lipid rafts. Within lipid rafts, the direct interaction of protease with the substrate may be unfavorable. Integrins through interaction with the disintegrin domains of the ADAM family members or directly with CD9 form a complex that is able to regulate the processing of membrane proteins such as growth factors, cytokines, and receptors by competing with the ADAM−tetraspanin interaction. Via the integrin−tetraspanin interaction, ADAM protease is sequestered outside of lipid rafts to allow for direct protease−substrate interaction.

Roles of ADAMs in Cytokine Biology

A number of substrates known in cytokine biology are listed in Table 1. TACE and ADAM 10 have been described as important sheddases for cytokines and their receptors, suggesting that specific targeting of these ADAMs provides a key for the modulation of cytokines *in vivo*. For ADAM 10, a role in chemokine fraktalkine CX3CL1 shedding was demonstrated (*56*). Another member of the cytokine family, the TNF-related activation-induced cytokine TRANCE, is cleaved by at least two different metalloproteases, one of which is MT-MMP1, but also by ADAM 19, as confirmed by peptide cleavage reactions (*57*).

Another ADAM with an apparent role in the immune system is ADAM 8 or CD156a, which was cloned in 1997 from a monocytic cell lineage (*58*). ADAM 8 has a very distinct expression pattern in macrophages, granulocytes, neurons, and reactive glia cells (*59*). In the latter cell types, expression of ADAM 8 is regulated by TNF-α in a dose-dependent manner (*59*), and may be part of a feedback regulatory loop, as depicted in Figure 3. Recently, two groups provided data on expression of ADAM 8. The metalloproteinase domain with its proteolytic function is not inhibited by TIMPs (tissue inhibitors of metalloproteinases) (*60*, *61*), and the physiological substrates of ADAM 8 are just beginning to be discovered. Like TACE and ADAM 10, ADAM 8 can process peptide substrates based on the precursor TNF-α. In addition, ADAM 8 may actually be a CD23 shedding enzyme (*24*). CD23 is a released from cells during an allergic response, and the soluble form stimulates IgE production in target cells (*62*). For years, pharmaceutical companies have been searching for the enzymes that are involved in CD23 shedding events as they present novel therapeutic targets for asthma and allergies. Thus, the possibility that ADAM 8 is a physiological CD23 sheddase is quite exciting and deserves further investigation. A specific inhibition of this activity would be important in the treatment of allergic reactions.

The finding that ADAM 8 may be an actual CD23 sheddase may explain the recent transgenic studies in which a soluble version of ADAM 8 is overexpressed using an α1-antitrypsin (AT) promoter (*63*). The soluble form of ADAM 8 is produced constitutively and results in the downregulation of L-selectin in neutrophils (PMN) compared to nontransgenic mice. In addition, the transgenic mice are more susceptible to an oxazolone-induced contact hypersensitivity reaction. These findings implicate ADAM 8 as being either directly or indirectly involved in L-selectin and CD23 shedding, although further experiments need to be carried

Table 1: ADAM Substrates Related to Cytokine Biology and Inflammatory Diseases

protease	substrate	effect of shedding (disease)	ref
ADAM 8	CD23	IgE response (allergy)	*24*
	TNFRI[a]	inflammation (asthma, TRAPS)	S. Naus *et al*., unpublished
	IL-1RII[a]	inflammation	*61*
ADAM 10	CX3CL1	chemotaxis inflammation (asthma, rhinitis)	*56*
ADAM 17	TNF-α	induction of apoptosis	*3, 4*
	TNFRI	TNF-α response (TRAPS)	*5, 16, 17, 22*
	TNFRII	inflammation	
	IL-1RII	IL-1β signaling	
ADAM 19	TRANCE	inflammation/ activated T cells	*57*

[a] Demonstrated for peptide cleavage.

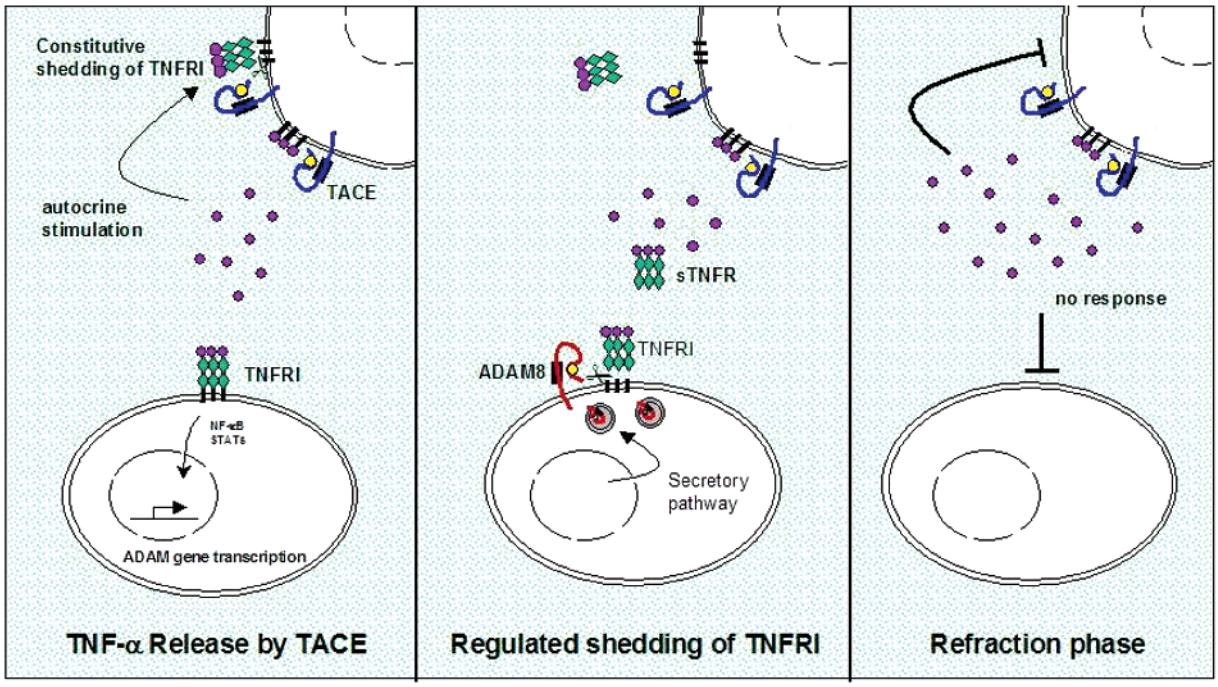

FIGURE 3: Hypothetical feedback loop formed by ADAM 8 and ADAM 17/TACE. In a TNF-α-producing cell (top right), constitutive shedding of TNF-α is achieved by TACE. Also, in an autocrine manner, TNF-α signaling is inhibited in the TNF-α-producing cell by shedding of TNFRI through TACE. In a paracrine manner, the target cell (middle panel) receives TNF-α via TNFRI and intracellular signaling (by NF-κB or STAT proteins) leads to transcriptional activation of the ADAM 8 gene. The corresponding protein is transported to the cell surface and activated in the secretory pathway. On the cell surface, ADAM 8 is able to cleave TNFRI, thereby desensitizing the target cells to high TNF-α concentrations. In this way, a dose-dependent cellular response is maintained.

out to confirm whether it is in fact a physiological convertase for either ligand.

On the basis of peptide cleavage results, TACE has a cleavage preference for TNFRII rather than for TNFRI. However, transcriptional regulation of ADAM 8 by TNF-α in several cell lines argues for a feedback mechanism in which ADAM 8 regulates the TNF-α susceptibility of cells by TNFRI cleavage. In accordance with this hypothesis, cleavage of the TNFRI peptide, but not of a TNFRII peptide, has already been proven by peptide analysis (S. Naus, D. Wildeboer, and J. W. Bartsch, unpublished data).

Other breakthroughs in the ADAM field, in addition to studies with ADAM 8, are findings reported with ADAM 12. ADAM 12 exists in membrane and soluble forms. *In vitro*, it processes insulin binding growth factors (IGFBP) 3 and 5 (*64*). IGFBPs bind IGF that is known to be a regulator of osteoarthritis and adipogenesis. Wewer *et al*. (*65*) overexpressed the soluble form of ADAM 12 under the control of the muscle creatine kinase promoter. These mice exhibit an accumulation of fat tissue around their skeletal muscle and develop both more abdominal and total body fat. Therefore, ADAM 12 could conceivably exert its influence on adipogenesis via regulation of circulating preadipocyte differentiation factors such as IGF, although other mechanisms at this point in time cannot be ruled out. In agreement with these findings, ADAM 12 deficient mice have reduced adipocytes and partial defects in myogenesis (*66*).

In addition to breakthroughs in the ADAM 12 field are recent reports identifying ADAM 33 as a potential "asthma gene". Genetic evidence points to ADAM 33, a locus on chromosome 20p13, which is linked to asthma and bronchial hyper-responsiveness (*67*). Curiously, most of the polymorphisms map to the cytoplasmic tail of ADAM 33 and not the catalytic domain, and uncertainties about whether inhibition of catalytic function could lead to therapeutic benefits exist. So far, substrate and inhibitor studies have just begun to be provided for ADAM 33 (*68*), but its importance in diseases such as asthma will help drive future studies.

ADAMs as Therapeutic Targets

In the area of drug development for metalloproteinases, there have been few successes with many failures. The most notable metalloproteinase inhibitor, captopril, is an angiotensin-converting enzyme inhibitor and is used for treatment of hypertension (*69*). For years, Roche and other labs have actively pursued inhibitors of the matrix metalloproteinase family whose members are similar to the ADAMs for treatments as diverse as cancer and arthritis (*70, 71*). Unfortunately, MMP inhibitors in the clinic have failed due to unwanted side effects such as tendonitis or fibroplasia (*72*) and for other unknown reasons. Recent advances in knockout studies suggest that a loss of MMP function may be detrimental. The MT1-MMP knockout mice, for example, exhibit an arthritis-like phenotype that is somewhat similar to that of fibroplasia that occurs clinically when cancer patients are treated with a broad spectrum MMP inhibitor such as Marimastat (*73*). In addition, inhibition of the gelatinases may exacerbate certain disease states such as cancer and chronic obstructive pulmonary disease (*74*). Recently, a Dupont/Bristol Myers TACE inhibitor that had reached phase II clinical trials for arthritis was discontinued for unknown reasons even though it did not exhibit an arthritis-like phenotype. A problem with TACE inhibition is only speculation, but may be due to the fact that its activity is too ubiquitous. Many shedding events would be inhibited by a TACE therapeutic. TACE therapy could be deleterious by leading to an accumulation of cell surface molecules such as precursor TNF. In transgenic animals carrying a mutated precursor TNF molecule that cannot be processed, localized sites of inflammation exist in the kidneys, liver, and vascular system. TACE inhibitors may be better suited for cancer therapy, where broad inhibition of ligand release is favorable.

For example, prevention of EGF receptor activation, through inhibition of TACE, ADAM 10, or ADAM 12, could lead to treatments for cardiac hypertrophy (*8*), cancer (*75*−*77*), and asthma (*78*). The release of EGF ligand family members such as TGF-α, amphiregulin, HB-EGF, betacellulin, and EGF as mediated by TACE and ADAM 10 is thought to occur through specific G-proteins (*10, 11*). One could envision a particular ADAM family member exerting different biological functions in different cell types through its activation by unique G-proteins. For example, ADAM 12 could be activated via melanocortin receptors since they play an important function in obesity (*79*). G-Protein activation through ligand binding could be a global mechanism by which different ADAM family members are turned on and are then involved in carrying out signaling events. The targeting of ADAM family members would therefore represent manipulation of G-protein-coupled signaling by downstream mediators.

Alternatively, some shedding events may occur via multiple ADAM family members that are specific to different cell types, and are "turned on" by specific activators, including G-proteins in that cell type.

Care must be taken when ascertaining what factors are actual mediators of disease states, and the actual ADAM family members that are responsible for shedding events. For example, a plausible mechanism for how EGF receptor activation is linked to cardiac problems could also be considered through effects on IL6 receptor (IL6R) shedding. IL6 receptor shedding is a metalloproteinase-dependent event, and activation of the stat pathway is known to occur via a complex of the soluble IL6 receptor and IL6 binding to GP130 (*80*). What is also known is that activation of the stat pathway in cardiomyocytes, through effectors such as G-protein agonists, leads to cell changes consistent with cardiac hypertrophy (*81*). In fact, constitutive activation of GP130 by overexpression of soluble IL6 and IL6R in transgenic mice causes myocardial hypertrophy (*82*). As there is cross talk between the EGF receptor and IL6 receptor in certain cell types, such that neutralizing antibodies to IL6 also prevent the EGF receptor from becoming activated (*83*), the mechanism by which metalloproteinase inhibitors prevent cardiac hypertrophy through a G-protein-coupled response could be IL6/IL6R activation of GP130. Therefore, the rationale for why a metalloproteinase inhibitor prevents cardiac hypertrophy (*8*) is possibly the fact that it is inhibiting IL-6R shedding and not HB-EGF release.

Recently, an essential role for ADAM 19 in congenital heart disease (a very common form of human birth defects) was defined by Blobel and co-workers. ADAM 19 deficient mice exert ventricular septal defects (*84*). ADAM 19 does not appear to process HB-EGF. Therefore, the defects probably represent shedding defects of other cell surface molecules.

Concluding Remarks

ADAM family members represent a unique class of enzymes that may play multiple roles in cell signaling, particularly in carcinogenesis and in diseases involving cytokine activation. The multifaceted nature of the ADAMs in terms of their structural domains suggests that they are not solely proteases with a given proteolytic function but also, more importantly, part of signaling pathways associated with G-protein-coupled responses. The functions of the tetraspanins and their interplay with both integrins and ADAMs make them molecular targets of great interest. An understanding of all steps required for G-protein-coupled activation is still elusive and stimulates this field of research.

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