

Chapter 2

STUDIES FROM ADAM KNOCKOUT MICE

Keisuke Hoiruchi and Carl P. Blobel

Arthritis and Tissue Degeneration Program and Cell Biology Program, Hospital for Special Surgery at Weil Medical College of Cornell University, New York, NY, USA

Abstract: ADAMs are membrane anchored glycoproteins that contain a disintegrin and metalloprotease domain. This chapter will focus on recent insights that have emerged from studies of “knockout” mice for ADAM proteases that are widely expressed or at least expressed in a variety of different cells and tissues (ADAMs 8, 9, 10, 12, 15, 17 and 19). These studies have shown that ADAM10 is important for signaling via the cell surface receptor Notch during development, while ADAM17 is critical for the development of the lung, epithelial structures and semilunar heart valves because of its role in the functional activation of ligands of the epidermal growth factor receptor. ADAM19 is essential for proper development of heart valves and the ventricular septum, although the underlying mechanism remains to be established. On the other hand, ADAMs 8, 9, 12 and 15 are dispensable for normal development and adult life in mice, at least under laboratory conditions. However, ADAM15 has a critical role in pathological neovascularization, making it a potential target for the design of inhibitors of angiogenesis. The availability of viable knockout mice for several widely expressed ADAM proteases sets the stage for a more comprehensive analysis of potential functions of these proteins in physiological and pathological processes. Furthermore, in light of the essential roles of ADAMs 10, 17 and 19 in development, it will be interesting to generate conditional knockout mice in order to evaluate the function of these proteases in adult animals.

Key words: ADAMs, knockout mice, metalloprotease-disintegrins, protein ectodomain shedding, Notch, EGFR, heart development, angiogenesis.

1. INTRODUCTION

ADAMs are a family of membrane anchored glycoproteins that have important functions in fertilization, neurogenesis, heart development, and in the ectodomain shedding of a number of membrane proteins, including the

pro-inflammatory cytokine TNF α , and ligands of the epidermal growth factor receptor (EGFR) (for recent reviews, see Kheradmand and Werb 2002; Primakoff and Myles 2000; Schlondorff and Blobel 1999; Seals and Courtneidge 2003; White 2003). A typical ADAM has an N-terminal pro-domain, followed by a metalloprotease domain, disintegrin domain, cysteine-rich region, EGF-repeat, transmembrane domain and cytoplasmic domain (Schlondorff and Blobel 1999; Wolfsberg *et al.* 1995a; Wolfsberg *et al.* 1995b). The first recognized ADAMs were the α and β subunit of the heterodimeric sperm surface protein fertilin, which is essential for fertilization in mice (Blobel *et al.* 1990; Blobel and White 1992; Cho *et al.* 1998; Primakoff *et al.* 1987; Wolfsberg *et al.* 1993). To date, over 30 ADAMs have been identified in a variety of species ranging from humans to mice, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Schizosaccharomyces pombe* (a continuously updated list of ADAMs can be found at: www.people.virginia.edu/~7Ejw7g/Table_of_the_ADAMs.html). These ADAMs have been found in several different ways. ADAM10 (Kuzbanian) emerged from a genetic screen for novel genes with a role in the Notch signaling pathway or in neuronal extension in *Drosophila melanogaster* (Fambrough *et al.* 1996; Rooke *et al.* 1996), while a biochemical purification of the TNF α converting enzyme activity resulted in the identification of ADAM17/TACE (Black *et al.* 1997; Moss *et al.* 1997). The majority of ADAMs were found in PCR screens with degenerate primers that were designed to amplify cDNAs for disintegrin- or metalloprotease domains (see, for example Cai *et al.* 1998; Kratzschmar *et al.* 1996; Weskamp and Blobel 1994; Weskamp *et al.* 1996; Yagami-Hiromasa *et al.* 1995), while more recently additional ADAMs have been identified through the mouse and human genome sequencing project. Because only relatively minor gaps remain in the human and mouse genome sequences, it now appears likely that most, if not all, mouse and human ADAMs have been identified.

Predictions regarding the function of ADAMs that were not identified in a functional or genetic screen are mainly based on what is known about the role of their different protein domains in other ADAMs or in related proteins found in snake venom. The disintegrin domain of ADAMs, for example, is related to snake venom integrin ligands called disintegrins (Huang *et al.* 1987; Niewiarowski *et al.* 1994). Therefore “orphan” ADAMs, that is ADAMs of unknown function, might have roles in cell-cell or cell-matrix interactions (Blobel *et al.* 1992; White 2003). Furthermore, about half of the currently known ADAMs contain a catalytic site consensus sequence (HEXXH) in their metalloprotease domain (Jongeneel *et al.* 1989; Stocker *et al.* 1995), and are therefore predicted to be catalytically active. The remaining ADAMs lack this consensus sequence, presumably do not possess catalytic activity and are therefore not included in this book on ADAM

proteases. Most ADAMs also contain putative cytoplasmic signaling motifs, such as proline-rich SH3-ligand domains and potential phosphorylation sites (Seals and Courtneidge 2003; Weskamp *et al.* 1996), raising the possibility that interactions with cytoplasmic proteins might regulate the function of ADAMs, or that ADAMs might have a role in intracellular signaling.

Following the identification of novel ADAM proteases, a number of different approaches were taken to elucidate their function, including purification and biochemical characterization of catalytically active enzymes (see, for example, Chesneau *et al.* 2003; Howard *et al.* 1996; Howard *et al.* 2001; Loechel *et al.* 1998; Roghani *et al.* 1999; Schlomann *et al.* 2002; Zou *et al.* 2004), evaluation of their potential role in cell adhesion and signaling (reviewed in White 2003), and studies of their expression patterns (see, for example Cai *et al.* 1998; Horiuchi *et al.* 2003; Kelly *et al.* 2004; Weskamp *et al.* 2002; Zhou *et al.* 2004). These approaches have provided much needed information about candidate substrates of catalytically active ADAMs, their possible role in cell adhesion, and about the cells and tissues in which they might have physiologically or pathologically relevant function. This has led to hypotheses about the roles of novel ADAMs which can be tested in a physiologically relevant model system. “Knockout” mice provide a particularly attractive model system for this purpose, and have the additional benefit that they can also deliver unexpected insights into the role of a given protein in development and disease. In this chapter, we summarize the findings that have emerged to date from studies of knockout mice for catalytically active ADAM proteases that are widely expressed or expressed in several different types of cells and tissues (ADAMs 8, 9, 10, 12, 15, 17 and 19, see Figure 1 for a dendrogram depicting the degree of sequence similarity among these 7 ADAMs and ADAM28).

2. ADAM8 (MS2, CD156a)

ADAM8 was originally identified in a macrophage cell line and named MS2 (Setoguchi *et al.* 1989). Expression of this gene was also found in central nervous system, bone cells, thymus, and lymphatic vessels. These observations and recent biochemical studies indicate possible roles of ADAM8 in inflammatory responses, neuropathology and bone metabolism. However, a gene targeting of ADAM8 revealed no apparent anomalies during development and in adult homeostasis. See also Chapter 3.

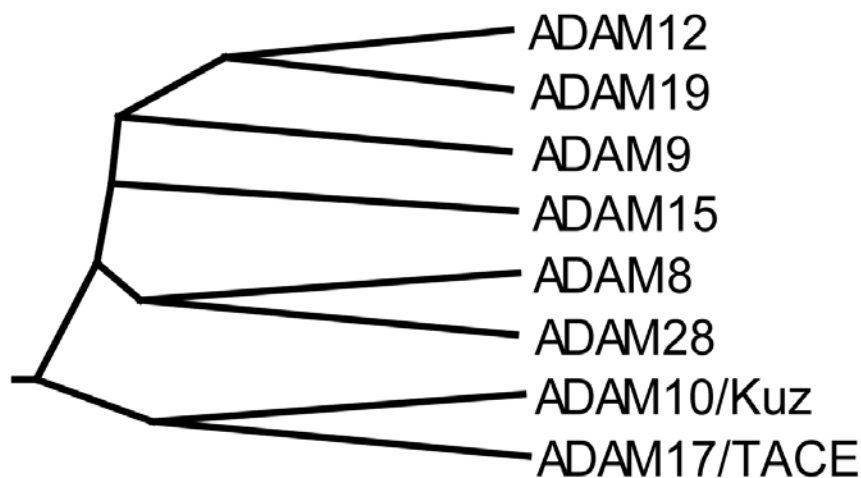


Figure 1. Dendrogram depicting the relative sequence relationship among catalytically active and widely expressed ADAMs in mice.

2.1 Expression pattern and putative functions of ADAM8 in immune system and neuropathology

ADAM8 was identified from a subtractive hybridization between cDNA from a macrophage cell line and a non-macrophage cell line (Setoguchi *et al.* 1989). Subsequent studies showed that ADAM8 is also expressed in human immune cells except for T-cells, and that the expression in macrophages is upregulated by macrophage stimulators (Yoshida *et al.* 1990; Yoshiyama *et al.* 1997). In adult mice, ADAM8 expression was also observed in the central nervous system and found to be induced by TNF α (Schlomann *et al.* 2000). At early developmental stages, prominent expression was found in extraembryonic tissues, while at later stages expression of ADAM8 was seen in several organs and tissues including the gonadal ridge, thymus, developing cartilage or bone, brain and spinal cord and in the mesenchyme in close proximity to developing blood vessels and lymphatic vessels (Kelly *et al.* 2004).

Recent biochemical studies showed that ADAM8 is catalytically active and is capable of processing myelin basic protein and peptides derived from the membrane-proximal region of several membrane bound molecules, including interleukin-1 receptor, kit ligand-1 (KL-1), amyloid precursor protein (APP), and CD23, a low affinity IgE receptor (Amour *et al.* 2002;

Fourie *et al.* 2003). Promoter studies have identified lipopolysaccharide and interferon- γ response elements in the 5' region of the ADAM8 gene (Kataoka *et al.* 1997).

Based on these observations, several studies aimed at uncovering a possible involvement of ADAM8 in inflammatory responses and neuropathological processes have been reported. Transgenic mice expressing the ectodomain of ADAM8 under the control of the $\alpha 1$ -antitrypsin promoter showed a potential role of ADAM8 in leukocyte infiltration (Higuchi *et al.* 2002). Experiments in Wobbler mice, which have an accelerated course of neurodegeneration, showed an increase in ADAM8 expression in activated glial cells (astrocytes and activated microglia), suggesting that ADAM8 has a role in pathological neuron-glia interactions (Schlomann *et al.* 2000). It was also reported that ADAM8, but not ADAM17 or ADAM10, is responsible for the shedding of membrane bound form of CHL1, close homologue of L1, which is thought to have important roles in the nervous system (Naus *et al.* 2004). The shed form of this molecule is upregulated in the brain extracts of Wobbler mice, suggesting a possible involvement of ADAM8 in the pathology of neurodegeneration.

ADAM8 expression was also found in osteoclasts and shown to have a stimulatory effect on osteoclast differentiation (Choi *et al.* 2001); this was further supported by the observation that ADAM8 is found upregulated in the tissue surrounding loosened hip prosthesis in human patients (Mandelin *et al.* 2003). Finally, recent studies with a mouse model of asthma indicated a possible role of ADAM8 in the pathology of asthma (King *et al.* 2004).

2.2 *Adam8*^{-/-} mice are viable and fertile with no apparent anomalies

ADAM8 gene-targeted mice were generated by replacing the coding sequence for the catalytic and disintegrin domain with a cassette containing the β -galactosidase and neomycin genes (Kelly *et al.* 2004). Both male and female adult *Adam8*^{-/-} mice are viable and fertile, producing litters at a frequency and size comparable to that of wild type controls. The appearance and behavior of *Adam8*^{-/-} mice is indistinguishable from that of their wild type and heterozygous littermates. Close histopathological examination, especially of the tissues where ADAM8 expression is high, did not reveal any evident anomalies. Although the expression pattern of ADAM8 during embryogenesis indicated a possible role in lymphangiogenesis, immunostaining with Prox1, a marker for lymphatic endothelial cells did not reveal any defects in lymphatic development. Bone morphology was also studied to address potential defects in either osteoclast or osteoblast development, but there were no apparent differences between *Adam8*^{-/-}

animals and wild type controls. Furthermore there were no significant changes in the differential blood counts, arguing against an essential and general function for ADAM8 in immune cell development. Thus, targeted inactivation of ADAM8 in mice did not lead to any overt defects in development, adult survival or fertility. These results demonstrate that ADAM8 is not essential for normal development and adult homeostasis. However, shedding of CHL1, which is related to the adhesion protein L1, is significantly decreased in brain extracts from *Adam8*^{-/-} mice compared to wild type animals (Naus *et al.* 2004). Further studies, including functional challenges of the immune system, as well as studies of neurodegeneration or bone metabolism in *Adam8*^{-/-} mice may help reveal the functions of this gene *in vivo*.

3. ADAM9 (MDC9, Meltrin γ)

ADAM9 was initially identified by PCR from mouse tissue and a mouse muscle cell line (Weskamp and Blobel 1994; Weskamp *et al.* 1996; Yagami-Hiromasa *et al.* 1995). ADAM9 is catalytically active (Roghani *et al.* 1999), and has been suggested to have a role in ectodomain shedding of heparin binding EGF-like growth factor (HB-EGF) and of the amyloid precursor protein (APP), a cell surface protein with a critical role in the pathogenesis of Alzheimer's diseases. However, cells derived from *Adam9*^{-/-} mice have no detectable defects in processing HB-EGF or APP, arguing against a major role of ADAM9 in the cleavage of these molecules in mice. *Adam9*^{-/-} mice are viable and fertile, and do not display any evident pathological phenotypes. See also Chapter 4.

3.1 Expression pattern and putative functions of ADAM9 as HB-EGF and APP sheddase

ADAM9 was first identified in a search for novel members of the ADAM gene family by degenerative PCR (Weskamp and Blobel 1994; Yagami-Hiromasa *et al.* 1995). It is ubiquitously expressed in adult tissues including the heart, brain, placenta, lung, skeletal muscle, digestive system and reproductive system (Weskamp *et al.* 1996). ADAM9 is also widely expressed during development, with the most prominent expression in the developing mesenchyme, heart and brain.

Overexpression of ADAM9 in Vero-H cells reportedly results in increased shedding of HB-EGF, suggesting that ADAM9 might have an important role in this process (Izumi *et al.* 1998). However this activity was

not observed in subsequent studies done by different groups (Hinkle *et al.* 2004; Prenzel *et al.* 1999; Sahin *et al.* 2004; Weskamp *et al.* 2002). ADAM9 is one of several ADAMs that has been suggested to function as an α -secretase for APP (also see Section 4.3 and 7.2) (Asai *et al.* 2003; Buxbaum *et al.* 1998; Koike *et al.* 1999; Lammich *et al.* 1999). The high expression of ADAM9 in the hippocampus in the brain would be consistent with this notion (Weskamp *et al.* 2002). Furthermore, ADAM9 has been shown to interact with integrins, including $\alpha v \beta 5$ and $\alpha 6 \beta 1$ (Nath *et al.* 2000; Zhou *et al.* 2001).

Interestingly, several studies have indicated ADAM9 as a pro-oncogenic factor, although the actual effects of ADAM9 in oncogenesis and metastasis are still not clear. An upregulation of ADAM9 was found in pancreatic ductal adenocarcinoma, hepatocellular carcinoma and breast cancer (Grutzmann *et al.* 2003; Grutzmann *et al.* 2004; Le Pabic *et al.* 2003; O'Shea *et al.* 2003; Tannapfel *et al.* 2003), and overexpression of ADAM9 in non-small lung cancer cells was shown to correlate with brain metastasis in a mouse model (Shintani *et al.* 2004). These observations indicate a possible involvement of ADAM9 in the pathogenesis in these tumors.

3.2 Mice lacking ADAM9 have no evident major abnormalities

Mice lacking ADAM9 were generated by gene targeting within the second exon, and the absence of ADAM9 in fibroblasts from *Adam9*^{-/-} embryos was confirmed by Western blot (Weskamp *et al.* 2002). Despite the ubiquitous expression pattern of ADAM9 during development and in adult tissues, *Adam9*^{-/-} mice develop normally and are viable and fertile, and there was no significant difference in the mortality rate between *Adam9*^{-/-} mice and wild type controls over the course of 2 years. Histopathological analysis of the organs from *Adam9*^{-/-} mice did not reveal any apparent abnormalities, and differential blood counts and blood chemistry were all within normal range. Thus ADAM9 is not essential for normal development and homeostasis. The processing of HB-EGF in *Adam9*^{-/-} fibroblasts was comparable to that of wild type controls. Furthermore hippocampal neurons isolated from *Adam9*^{-/-} mice did not show detectable defects in the processing of APP at the metalloprotease-dependent α -secretase cleavage site, arguing against a critical role of ADAM9 as α -secretase. Thus ADAM9 is either not essential for the processing of APP or can be compensated by other sheddases such as ADAM10 and ADAM17. Further studies will be necessary to uncover possible functions of ADAM9 during development or in adult mice, and to address whether ADAM9 has a role in oncogenesis.

4. ADAM10 (Kuzbanian, MDM)

ADAM10 was originally purified as an activity that efficiently degrades myelin basic protein in brain extracts. Peptide sequences obtained from the purified protein later allowed cloning of ADAM10 cDNA (referred to as MADM, mammalian disintegrin-metalloprotease) from a bovine cDNA library (Howard *et al.* 1996). Subsequently it became clear that ADAM10 is the mammalian homologue of *Drosophila* Kuzbanian (Kuz), which in turn has an essential role in Notch/Delta signaling. In addition, ADAM10 is considered one of several candidate α -secretases for APP. A targeted mutation of ADAM10 in mice revealed that ADAM10 is indeed essential for Notch/Delta signaling in mammals, but not for the α -secretase dependent processing of APP. See also Chapter 10.

4.1 Expression pattern

A study of the expression pattern of ADAM10 during embryogenesis was done in chicks. ADAM10 was found to be expressed ubiquitously in embryos, including in the dermatome, myotome of the somites, epidermis, gut endoderm, epithelial tissues of the kidney, liver, heart, and neural crest cells (Hall and Erickson 2003). Western blot analysis showed that ADAM10 is also highly expressed in a variety of adult tissues (Sahin *et al.* 2004). In adult brain, ADAM10 was found to be widely expressed throughout the brain, which is consistent with a putative role of this gene as an α -secretase for APP (Karkkainen *et al.* 2000).

4.2 Controversies over the functions of ADAM10 in Notch/Delta signaling

Studies in *Drosophila* were the first to uncover a critical role for ADAM10 in neural cell fate decisions (Rooke *et al.* 1996). Rooke *et al.* showed that loss-of-function mutations in the Kuz gene (the *Drosophila* orthologue of ADAM10) result in defects resembling those caused by mutations in Notch, a membrane anchored receptor with important roles in cell fate decisions during development. The mechanism underlying Notch/Delta dependent cell fate decisions is now thought to be conserved from worms to mammals and in a wide variety of cells (Artavanis-Tsakonas *et al.* 1995; Artavanis-Tsakonas *et al.* 1999; Greenwald 1998).

Studies on the processing of Notch over the past few years revealed that Notch undergoes three proteolytic cleavages, at distinct cleavage sites termed S1, S2 and S3. Several lines of evidence indicate that proteolysis of

one of the extracellular cleavage sites, termed S2 site, is Kuz/ADAM10 dependent. Pan and Rubin demonstrated that Kuz functions upstream of Notch by showing that gain-of-function Notch is epistatic to coexpression of a dominant-negative form of Kuz (Pan and Rubin 1997). More recently, it has been shown that Kuz associates with Notch and that RNA interference against Kuz blocks S2 cleavage of Notch in flies (Lieber *et al.* 2002).

On the other hand, a study which aimed at identifying the protease responsible for S2 cleavage activity in mammalian cells in tissue culture found, quite unexpectedly, that ADAM17, but not ADAM10, is responsible for this activity (Brou *et al.* 2000). This group also demonstrated that differentiation of *Adam17*^{-/-} myeloid precursor cells to macrophages, a process that is known to be inhibited by Notch signaling, could be blocked by rescuing these cells with ADAM17. Consistent with these observations, mammalian cells lacking ADAM10 still exhibit S2 Notch cleavage activity *in vitro* (Mumm *et al.* 2000). However, the finding that *Adam17*^{-/-} mice do not show any apparent phenotype related to Notch or Notch ligand loss of function (see below), raises questions about how relevant ADAM17 is for Notch processing and activation during development *in vivo*. Thus much remains to be learned about the physiological roles of ADAM17 and ADAM10 in Notch/Delta signaling in mammals.

4.3 ADAM10 as a candidate for APP α -secretase

Intriguingly there is a strong resemblance between the biochemical processing of Notch and APP. Yet even though it appears clear that ADAMs have an important role in processing both proteins, the identity of the ADAM that is relevant for either process *in vivo* is not yet clearly defined. As in the case of Notch, APP is cleaved at several distinct sites by at least three different proteases, termed α -, β - and γ -secretase. Cleavage of APP by β - and γ -secretase gives rise to the A β peptide, which is a major component of amyloid plaques and is thought to have a critical role in the pathogenesis of Alzheimer's disease (Selkoe and Schenk 2003). Cleavage of APP by α -secretase occurs between the β - and γ - cleavage site, thus preventing the production of A β peptides. Therefore the α -secretase is considered to be a protective factor against Alzheimer's disease.

Along with ADAM17 and ADAM9, ADAM10 is thought to be a potential α -secretase (Kojro *et al.* 2001; Lammich *et al.* 1999; Lopez-Perez *et al.* 2001). Overexpression of ADAM10 in HEK cells leads to an increased α -secretase activity, and endogenous α -secretase activity in these cells is inhibited by introducing a dominant negative form of ADAM10 (Lammich *et al.* 1999). ADAM10 is widely expressed in the brain, including neurons, whereas expression of ADAM17 is more or less limited to the endothelia

and glia (Bernstein *et al.* 2003; Goddard *et al.* 2001; Karkkainen *et al.* 2000). The expression pattern in the brain is more consistent with a role for ADAM10 (and ADAM9) than ADAM17 as the APP α -secretase, although expression analysis is not a completely reliable indicator for where an enzyme might function (even low levels of an ADAM may be completely sufficient for its physiological activity). A recent study also showed that overexpression of ADAM10 in neurons alleviates amyloid plaque formation and hippocampal defects in an Alzheimer's disease mouse model (Postina *et al.* 2004). While this study suggests that increasing the activity of ADAM10 may be therapeutically desirable, it does not clarify the identity of the physiological α -secretase.

4.4 Other possible substrates of ADAM10

Apart from processing Notch/Delta and APP, ADAM10 has also been implicated in regulating neuronal repulsion through cleaving ephrinA2 (Hattori *et al.* 2000), and in the processing of various other cell surface molecules, including CD44 (Murai *et al.* 2004), type IV collagen (Millichip *et al.* 1998), L1 (Mechtersheimer *et al.* 2001), interleukin 6 receptor (Matthews *et al.* 2003), CXC chemokine ligand 16 (Abel *et al.* 2004; Gough *et al.* 2004), CX3C chemokine fractalkine (Hundhausen *et al.* 2003), neurotensin receptor-3 (Navarro *et al.* 2002), prion protein (Vincent *et al.* 2001), HB-EGF (Lemjabbar and Basbaum 2002; Schafer *et al.* 2004; Yan *et al.* 2002), betacellulin, EGF (Sahin *et al.* 2004), β -site amyloid precursor protein-cleaving enzyme 1 (Hussain *et al.* 2003). The physiological relevance of the processing of most of these candidate ADAM10 substrates during development *in vivo* remains to be determined.

4.5 Deletion mutation of ADAM10 leads to early embryonic lethality and multiple malformations which resemble those seen in the absence of Notch/Delta signaling

In light of the complexities surrounding the potential role of ADAM10, in the processing of Notch/Delta and APP, generation and analysis of *Adam10*^{-/-} mice were expected to provide important new insights into these issues. Gene targeting of ADAM10 was performed by inserting a neomycin cassette into the second exon of the ADAM10 gene (Hartmann *et al.* 2002). *Adam10*^{-/-} embryos were found to die around 9.5 dpc, with multiple malformations strikingly similar to that of a complex Notch deficiency as seen in presenilin1/presenilin2 or notch1/notch4 double mutant mice

(Herreman *et al.* 1999; Krebs *et al.* 2000). Consistent with these observations, the expression pattern of the genes involved in the Notch pathway, *dll-1*, one of the ligands of Notch, and *hes-5*, a transcription factor activated by Notch signaling, was severely disrupted. These findings further support the notion that, as in insects and worms, ADAM10, but not ADAM17, is essential for the Notch/Delta signaling during mammalian development.

It should be noted that the phenotype of *Adam10*^{-/-} embryos is more severe than that of presenilin1/presenilin2 double knockout mice, where cleavage dependent Notch/Delta signaling is thought to be blocked (Herreman *et al.* 1999; Herreman *et al.* 2000). This strongly suggests that ADAM10 also has additional roles in processing of ligands or receptors other than Notch or Delta during embryogenesis.

As mentioned above, the role of ADAM10 in APP cleavage remains a somewhat controversial issue. Studies using hippocampal neurons derived from *Adam9*^{-/-} mice revealed that absence of ADAM9 does not have a major impact on APP processing in these cells, while the absence of ADAM17 abolished regulated (PMA-stimulated), but not constitutive, α -secretase activity in mouse embryonic fibroblasts (Buxbaum *et al.* 1998; Weskamp *et al.* 2002). Due to the early embryonic lethality of *Adam10*^{-/-} mice at a stage preceding neuronal development, APP processing can only be analyzed in immortalized cell lines derived from *Adam10*^{-/-} embryos. The results might therefore not directly reflect the actual contribution of ADAM10 in APP cleavage in the brain. Nevertheless the data clearly showed that α -secretase activity was preserved in some *Adam10*^{-/-} cell lines. It remains to be determined whether there is compensation by, or redundancy between, different ADAMs or other enzymes in the processing of APP (Asai *et al.* 2003) or whether different ADAMs have major roles in APP processing in distinct cells and tissues. Since further studies of the role of ADAM10 during development and in adult mice are hampered by the early embryonic lethality of *Adam10*^{-/-} mice, it will be critical to generate mice carrying a conditional mutation in this gene to learn more about the function of ADAM10 during development and in adults, and in APP processing in the brain.

5. ADAM12 (Meltrin α)

ADAM12 was initially identified along with ADAM19 (meltrin β) and ADAM9 (meltrin γ) in myoblasts, where it was suggested to play a role in myoblast fusion during myoblast differentiation (Yagami-Hiromasa *et al.* 1995). A deletion mutation, however, did not lead to any overt defects,

including in muscle development and regeneration, arguing against an essential role for ADAM12 in muscle fusion or differentiation during mouse development. See also Chapter 6.

5.1 Expression pattern

In the early stages of murine development, ADAM12 transcripts are expressed in the condensed mesenchymal cells which later develop to skeletal muscle, bones and visceral organs (Kurisaki *et al.* 1998). ADAM12 transcripts in the myotome appear at 10.5-11.5 dpc, after myotube formation takes place. Although ADAM12 expression persists in the tendinous region of the muscle in later stages of development, the most prominent expression is found in the developing bone, especially in the periosteum and bone marrow (Kurisaki *et al.* 1998). ADAM12 was initially reported to have a very limited expression pattern in adult tissue, with high expression in bone (Yagami-Hiromasa *et al.* 1995). Due to its expression in bone and muscle cells, ADAM12 was thought to play a role in the fusion of myoblasts and osteoclasts. Later studies revealed that ADAM12 is also expressed quite ubiquitously in adult mice, and that osteoblasts are the major source of ADAM12 transcripts in the bone (Gilpin *et al.* 1998; Harris *et al.* 1997; Inoue *et al.* 1998).

5.2 Putative role of ADAM12 in myogenesis

During development myoblasts are known to fuse to form mature multinucleated myotubes. As mentioned above, ADAM12 was originally identified in a search for genes involved in muscle fusion by PCR with degenerate primers for conserved amino acids in the first recognized ADAMs, fertilin α and β , which were considered to be candidate fusion proteins at the time (we now know that fertilin α and β have an essential role in fertilization, but are not required for sperm-egg membrane fusion *in vivo*) (Primakoff and Myles 2002). Overexpression of a truncated form of ADAM12 lacking the pro- and metalloprotease domain in a myoblast-like cell line (C2 cells), significantly enhanced myoblast fusion *in vitro*, whereas C2 cells transfected with either full length ADAM12 or anti-sense RNA exhibited less membrane fusion. It was also shown that ADAM12 contains a sequence similar to the fusion peptides of paramyxoviruses (Yagami-Hiromasa *et al.* 1995).

A subsequent study demonstrated that an isoform of ADAM12 which lacks a transmembrane domain recruits host muscle cells into implanted human tumors in nude mice (Gilpin *et al.* 1998). Furthermore, the cytoplasmic domain of ADAM12 interacts with the muscle specific protein

α -actinin-2 and this interaction is essential for ADAM12 to promote myoblast fusion (Galliano *et al.* 2000). In addition, introduction of ADAM12 in a mouse model of muscular dystrophy (mdx mice) significantly alleviates the muscle pathology (Kronqvist *et al.* 2002). Finally, the expression of ADAM12 is localized to muscle satellite cells and is upregulated in regenerating and denervated muscle (Borneman *et al.* 2000).

Despite these studies, however, it remains to be determined what the exact role of ADAM12 in muscle development and regeneration is. First, although the removal of the prodomain is known to be important for generation of mature and catalytically active ADAM12 (Cao *et al.* 2002; Loechel *et al.* 1998; Yagami-Hiromasa *et al.* 1995), there is little evidence for the existence of an ADAM12 which lacks its pro- and metalloprotease domain (the truncated form used in *in vitro* myotube formation assays (Yagami-Hiromasa *et al.* 1995)). Second, although ADAM12-S, a soluble isoform which contains the metalloprotease domain, has been implicated in recruitment of myogenic cells into tumors (Gilpin *et al.* 1998), transgenic mice overexpressing ADAM12-S under control of the muscle creatine kinase promoter resulted in induction of adipogenesis but had no overt defect in muscle differentiation (Kawaguchi *et al.* 2002).

A recent study by Cao *et al.* prompted a different view on this issue. This study showed that while inhibition of the expression of ADAM12 was accompanied by lower expression of markers for both quiescent and differentiating cells, overexpression of ADAM12 induced a quiescent cell-like phenotype and did not stimulate differentiation, indicating that ADAM12 has a role, as a negative regulator, in cell fate decision in myoblast differentiation (Cao *et al.* 2003). This observation offers some reconciliation with the early study in which stable transfection of C2 cells with the full-length ADAM12 led to inhibition of myoblast fusion (Yagami-Hiromasa *et al.* 1995), and may provide an explanation for the phenotype of ADAM12-S transgenic mice and ADAM12 null mice (discussed below). However it does not lend support to the notion that ADAM12 participates in the process of myoblast fusion or contributes to muscle regeneration in dystrophic mice model as previously shown. Finally, ADAM12 has also been reported to have a role in the formation of multinucleated osteoclasts, which are formed by cell-cell fusion of mononuclear precursors (Abe *et al.* 1999).

5.3 Putative substrates and interacting molecules

As with other members of this gene family, ADAM12 has been implicated as a protease of several molecules, including Insulin-like growth factor binding protein-3 and -5 (Loechel *et al.* 2000; Shi *et al.* 2000),

oxytocinase (Ito *et al.* 2004) and HB-EGF (Asakura *et al.* 2002; Mori *et al.* 2003). However, considering the recent genetic and biochemical evidence pointing toward ADAM17 as the major sheddase for HB-EGF, the physiological relevance of ADAM12 in cleavage of HB-EGF remains to be determined (see Section 7.2)

Several molecules have also been shown to interact with the cytoplasmic domain of ADAM12. These include Src (Kang *et al.* 2000), Grb2 (Suzuki *et al.* 2000), α -actinin-1 (Cao *et al.* 2001), FISH (Abram *et al.* 2003), phosphatidylinositol 3-kinase (Kang *et al.* 2001) and PACSIN3 (Mori *et al.* 2003). The disintegrin and cysteine rich domain binds to integrin $\alpha 9 \beta 1$ and Syndecan-4 (Iba *et al.* 2000; Iba *et al.* 1999; Thodeti *et al.* 2003), respectively.

5.4 Removal of ADAM12 does not lead to overt anomalies in muscle

The initial identification of ADAM12 from muscle cells, and the functional studies with mutant forms of ADAM12 in these cells suggested that ADAM12 has an important role in myogenesis. To address this issue, *Adam12*^{-/-} mice were generated in which the first exon of the ADAM12 gene was replaced with a neomycin cassette. Unexpectedly, *Adam12*^{-/-} mice did not show any overt abnormalities, even though 30% less *Adam12*^{-/-} mice were born from crosses of heterozygous parents than expected. Because the ratio of offspring from matings of *Adam12*^{+/-} mice was Mendelian prior to birth, the missing 30% of mutant mice must have died several days after birth. The cause of this perinatal lethality remains to be determined (Kurisaki *et al.* 2003). Intriguingly, *Adam9/15/12*^{-/-} triple knock out mice are viable and fertile and their survival rate was comparable to that of wild type controls (Sahin *et al.* 2004). One possible explanation for this discrepancy is that there may have been differences in the genetic background of mice used in these different studies.

Close histological analysis of *Adam12*^{-/-} mice revealed subtle defects in the interscapular brown fat tissue, and in the neck and interscapular muscles of around 30% of the examined animals (Kurisaki *et al.* 2003). It remains to be determined why only the neck and interscapular region are affected by the loss of ADAM12, and whether these defects may be the cause for the 30% embryonic or perinatal lethality in *Adam12*^{-/-} mice. Muscle regeneration in *Adam12*^{-/-} mice was comparable to wild type controls, and when mice carrying the mdx mutation, which results in muscular dystrophy, were bred with *Adam12*^{-/-} mice, no aggravation of muscle degeneration was seen in *Adam12*^{-/-}-mdx animals. Taken together, removal of ADAM12 did not lead to any major defects, including in muscle development and regeneration. On

the other hand, phorbol ester-stimulated shedding of HB-EGF was significantly reduced in *Adam12*^{-/-} fibroblasts (Kurisaki *et al.* 2003). This is in contrast with the results from Sahin *et al.* in which PMA-stimulated and constitutive shedding of HB-EGF was not strongly affected in *Adam9/15/12*^{-/-} triple knockout fibroblasts. Nevertheless, *Adam12*^{-/-} mice do not reveal any heart defects resembling those observed in HB-EGF null mice, while *Adam17*^{-/-} mice have similar heart defects as *Hb-egf*^{-/-} mice (see Section 7.2), suggesting that ADAM12 is not essential for HB-EGF shedding and activation during heart development.

Given that ADAM12 may be involved in differentiation of self-renewing satellite cells *in vivo* (Cao *et al.* 2003), this provides a possible explanation for the lack of apparent muscle defects in *Adam12*^{-/-} mice and ADAM12 transgenic mice. Based on the observations by Cao *et al.*, only high expression of ADAM12 prior to muscle differentiation can drive myoblasts toward a quiescent state. Hence either removal of ADAM12 or overexpression of ADAM12 in differentiated myofibers (with muscle specific creatine kinase promoter as done in previous studies) may not have a major impact on this event (Cao *et al.* 2003). Nevertheless, the lack of apparent muscle phenotype in *Adam12*^{-/-} mice argues against an essential role for ADAM12 in myoblast fusion / differentiation (Yagami-Hiromasa *et al.* 1995). One possible explanation is that this result is due to compensation or redundancy with other ADAMs, even though neither *Adam9/12/15/17* nor *Adam9/12/15/19* quadruple mutant mice revealed apparent muscle defects during development (Sahin *et al.* 2004; Horiuchi *et al.*, manuscript in preparation). Further studies will be necessary to understand the role of ADAM12 in muscle differentiation and regeneration.

6. ADAM15 (MDC15, Metarginin)

Human ADAM15 was first discovered in a screen for novel ADAMs by PCR (Kratzschmar *et al.* 1996), and was named metarginin because it carries an RGD sequence in a similar position as snake venom disintegrins (Kratzschmar *et al.* 1996). However, the mouse and rat orthologues of ADAM15 lack an RGD sequence (Bosse *et al.* 2000; Lum *et al.* 1998), arguing against a conserved role of ADAM15 as ligands for RGD-binding integrins in these two rodents.

6.1 Expression pattern in vascular cells and putative function in angiogenesis

ADAM15 expression can be detected in most tissues in developing and adult mice and in specific regions of the brain and spinal cord (Bosse *et al.* 2000; Horiuchi *et al.* 2003; Lum *et al.* 1998). During development the highest levels of expression are observed in vascular cells, endocardial cells and in hypertrophic cells in developing bone (Horiuchi *et al.* 2003). The expression in developing vessels peaks at 12.5-14.5 dpc and subsequently subsides (unpublished observation). After birth, high expression of ADAM15 in the vasculature was seen in endothelial cells in the retina of animals subjected to a mouse model of retinopathy of prematurity. ADAM15 is also highly expressed in human umbilical vascular endothelial cells and vascular smooth muscle cells (Herren *et al.* 1997; Horiuchi *et al.* 2003), however it is not as highly expressed in normal vessels in adults (unpublished observation; Herren *et al.* 1997).

In accordance to its expression pattern in vasculature, several studies have indicated a possible involvement of ADAM15 in angiogenesis. Expression of ADAM15 has been shown to be upregulated in atherosclerotic lesions (Al-Fakhri *et al.* 2003; Herren *et al.* 1997). It has also been shown that ADAM15 colocalized with VE-cadherin, an endothelial cell specific cell-cell adhesion molecule, and the surface expression of ADAM15 was driven by this molecule (Ham *et al.* 2002).

Human ADAM15 contains an RGD sequence and has been shown to interact with $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins (Nath *et al.* 1999; Zhang *et al.* 1998), both of which are known to play crucial roles in angiogenesis (Eliceiri and Cheresh 2000; Hynes 2002), while mouse ADAM15 does not contain this sequence. It is therefore possible that human ADAM15 has different functions *in vivo* than mouse ADAM15 because it can interact with these two integrins. Interestingly, a recent study showed that the disintegrin domain of human ADAM15, which contains the RGD sequence, has a anti-oncogenic activity through suppressing tumor angiogenesis (Trochon-Joseph *et al.* 2004). Both mouse and human ADAM15 have been shown to interact with $\alpha 9\beta 1$ integrin in an RGD-independent manner, as is the case with other ADAMs, such as ADAM1, 2, 3, 9 and 12.

Although ADAM15 carries a catalytic site consensus sequence for metalloproteases, little is currently known about its catalytic activity. So far, ADAM15 has been implicated in processing CD23 (Fourie *et al.* 2003), type IV collagen, gelatin (Martin *et al.* 2002), TGF α and amphiregulin (Schafer *et al.* 2004) *in vitro*. However, ADAM15 null mice do not show any EGFR related developmental defects (described below) and fibroblasts from *Adam9/12/15*^{-/-} embryos are still capable of processing TGF α and

amphiregulin (Sahin *et al.* 2004). The biological relevance of these observations therefore remains to be clarified.

Several cytoplasmic proteins have also been shown to interact with the cytoplasmic domain of ADAM15. These include Src family protein kinases, the adaptor protein Grb2 (Poghosyan *et al.* 2002), SH3PX1 and endophilin I (Howard *et al.* 1999).

6.2 *Adam15*^{-/-} mice are viable and fertile but have decreased pathological neovascularization in a mouse model for proliferative retinopathy

In order to generate *Adam15*^{-/-} mice, a targeted mutation was introduced into the ADAM15 gene by replacing the exon carrying the initial methionine with a neomycin cassette. When heterozygous *Adam15*^{+/-} mice of mixed genetic background (129/SvJ, C57Bl/6J) or of inbred genetic background (129/SvJ or C57Bl/6J) were mated, the genotype of the offspring followed a Mendelian distribution pattern, regardless of the background, and *Adam15*^{-/-} mice were indistinguishable from control littermates in appearance and their behavior during routine handling. Matings of *Adam15*^{-/-} mice resulted in viable and healthy litters with normal litter sizes. Histopathological analysis, especially in the tissues where ADAM15 expression is high, as well as clinical chemistry analysis of serum and differential blood count revealed no apparent anomalies. Furthermore, no significant difference in mortality or morbidity between *Adam15*^{-/-} and wild type control mice was seen over two years. These observations show that ADAM15 is not essential for either development or adult homeostasis.

Because ADAM15 is highly expressed in developing blood vessels, *Adam15*^{-/-} mice were subjected to a mouse model of retinopathy, in which neovascularization in the retina is induced by relative hypoxia. *Adam15*^{-/-} mice showed remarkably fewer neovascularization tufts compared to wild type controls, indicating a possible involvement of ADAM15 in the pathology of proliferative retinopathy. Furthermore, growth of heterotopically injected tumors was decreased in *Adam15*^{-/-} mice compared to wild type controls, which is consistent with a role for ADAM15 in neovascularization, even though there was no apparent decrease in the microvessel density in the tumors from *Adam15*^{-/-} mice compared to that of wild type controls. This could be explained by defects in certain aspects of neovascularization, such as a delay in the initiation of new vascular sprouts or in the growth rate of new vessels. To further explore these possibilities, endothelial cells and aortae isolated from *Adam15*^{-/-} and wild type control mice have been examined. However the cells and tissues from *Adam15*^{-/-}

behaved identically to that of wild type controls in *in vitro* studies, including studies of proliferation, migration and tube formation of endothelial cells, and aortic explant sprouting assays (unpublished observation). Further studies are in progress to elucidate the mechanism underlying the role of ADAM15 in pathological neovascularization.

As described, $\alpha 9\beta 1$ integrin can interact with several ADAMs including ADAM9, 12 and 15. Mice lacking $\alpha 9\beta 1$ integrin develop a chylothorax after birth and die of respiratory failure (Huang *et al.* 2000). However single knockout mice for ADAMs 9, 12 or 15, or ADAM9/15 double mutant or even ADAM9/12/15 triple mutant mice are viable and fertile and do not display a chylothorax or respiratory failure (Sahin *et al.* 2004). The biological significance of the interaction between ADAMs and $\alpha 9\beta 1$ integrin therefore remains to be determined.

7. ADAM17 (TACE)

ADAM17 can currently be considered the best-characterized ADAM besides ADAM10. Originally it was identified as an enzyme responsible for the cleavage of TNF α (Black *et al.* 1997; Moss *et al.* 1997), although further studies uncovered more diverse functions than initially expected. Gene targeting resulted in perinatal lethality with multiple defects in various organs which closely resemble those seen in mice lacking the EGFR, TGF or HB-EGF. See also Chapter 8.

7.1 Prominent role of ADAM17 as a multiple sheddase

ADAM17 is the first ADAM for which a role as a “sheddase” was clearly established. It was initially identified in a search for the TNF α converting enzyme (TACE), i.e. the enzyme that releases TNF α from cells (Black *et al.* 1997; Moss *et al.* 1997). ADAM17 is widely expressed with high levels of expression in the heart, placenta, testis, ovary, lung and spleen (Black *et al.* 1997; Sahin *et al.* 2004).

Apart from TNF α , a substantial number of other membrane proteins have been identified as substrates for ADAM17, including several EGFR ligands (HB-EGF, amphiregulin, TGF, epiregulin) (Sahin *et al.* 2004; Sunnarborg *et al.* 2002), p75 TNFR (Peschon *et al.* 1998a), IL6R (Matthews *et al.* 2003), APP (Buxbaum *et al.* 1998; Slack *et al.* 2001), MUC1 (Thathiah *et al.* 2003), growth hormone binding protein (Zhang *et al.* 2000), L-selectin (Condon *et al.* 2001; Peschon *et al.* 1998a), Fractalkine (CX3CL1) (Garton *et al.* 2001), collagen XVII (Franzke *et al.* 2004), prion protein (Vincent *et al.* 2001), CD40 (Contin *et al.* 2003), PAR1 (Contin *et al.* 2003), c-Kit (Cruz *et al.*

2004), VCAM-1 (Garton *et al.* 2001), p75 neurotrophin receptor (Weskamp *et al.* 2004) and ErbB4 (Rio *et al.* 2000). Not surprisingly, ADAM17 has been implicated in a variety of physiological and pathological processes, including tumor migration and proliferation (Borrell-Pages *et al.* 2003; Gschwind *et al.* 2003; Hart *et al.* 2004; Schafer *et al.* 2004), arthritis and inflammation (Newton *et al.* 2001; Ohta *et al.* 2001; Patel *et al.* 1998). Furthermore, it has also been shown that ADAM17 interacts with several other molecules, including $\alpha 5 \beta 1$ integrin (Bax *et al.* 2004), MAD2 (Nelson *et al.* 1999), PTPH1 (Zheng *et al.* 2002) and SAP97 (Peiretti *et al.* 2003). However, the physiological relevance of these interactions remains to be established.

7.2 Inactivation of ADAM17 leads to perinatal lethality with multiple defects resembling those seen in *Egfr*^{-/-}, *Tgfa*^{-/-} and *Hb-egf*^{-/-} mice

In order to inactivate *Adam17*, the exon carrying the Zn^{2+} binding catalytic site was replaced with a neomycin cassette by homologous recombination (Black *et al.* 1997). T-cells derived from the mutant animals showed a significant reduction in TNF release and an increase in surface TNF α expression compared to the wild type controls. Initially, there was some concern about this gene targeting strategy because the resulting mutant form of ADAM17 lacking the catalytic site could conceivably have a dominant negative effect. However all data available to date suggests that this mutation leads to a loss of function instead of a dominant negative effect (see Schlondorff and Blobel 1999 for discussion).

Analysis of progeny derived from matings of *Adam17*^{+/-} mice revealed that most *Adam17*^{-/-} mice die between 17.5 dpc and the first day of birth. In light of the predicted role of ADAM17 in the processing of TNF α (at the time, TNF α was the only known substrate for ADAM17), this severe phenotype was quite unexpected because mice lacking TNF α or its receptors are overtly normal (Marino *et al.* 1997; Pasparakis *et al.* 1996; Peschon *et al.* 1998b). *Adam17*^{-/-} mice have open eye lids resulting from a failure of eyelid fusion, lack a conjunctival sac, and have thinned corneas and several epidermal and hair defects. These defects in the eye, hair and skin are reminiscent of those seen in mice bearing a disruption of the gene for TGF α . Additional defects were observed in the epithelial maturation of multiple organs, including the intestine, lung, nonglandular stomach, thyroid, parathyroid and salivary gland, and in the spongiotrophoblast layer of the placenta. The epithelial defects are similar to those described in mice lacking the EGFR. Since all ligands of EGFR, including TGF α , HB-EGF, epiregulin,

amphiregulin, EGF and betacellulin, are synthesized as membrane bound precursors and are subsequently released from the cell surface (Harris *et al.* 2003), this raised the possibility that ADAM17 might be responsible for the processing of one or more of these ligands in addition to TNF α .

Consistent with this notion, the processing of TGF α was indeed absent in the fibroblasts derived from *Adam17*^{-/-} mice (Black *et al.* 1997). Moreover an anatomical analysis of *Adam17*^{-/-} mice revealed multiple defects in the heart, especially in valvulogenesis, and in the lung, indicating that these defects in the major organs could be the cause of the perinatal lethality of *Adam17*^{-/-} mice (Shi *et al.* 2003; Zhao *et al.* 2001). These findings are especially interesting in the light of the recent study showing very similar defects in the heart and lung in HB-EGF null mutant mice (Iwamoto *et al.* 2003; Jackson *et al.* 2003). These observations, along with emerging *in vitro* data, provide genetic and biochemical evidence that ADAM17 is also a major sheddase for HB-EGF. Although several ADAMs, including ADAM9, ADAM10 and ADAM12 have also been shown to take part in the processing of HB-EGF *in vitro* as described, the knockout mice for ADAMs 9 and 12 do not show a phenotype similar to that of HB-EGF (*Adam10*^{-/-} mice die too early to allow an evaluation of heart valve development). Thus, even though it cannot be excluded that proteases other than ADAM17 might have a more prominent role in shedding HB-EGF under certain conditions (such as in pathological conditions e.g. cancer or wound healing), ADAM17 appears to be the physiologically relevant sheddase for HB-EGF during heart development. As for other EGFR ligands, ADAM17 has also been implicated in the cleavage of amphiregulin and epiregulin, further supporting the initial hypothesis that ADAM17 is important for activating several ligands of the EGFR (Gschwind *et al.* 2003; Hinkle *et al.* 2004; Sahin *et al.* 2004).

Due to the perinatal lethality of *Adam17*^{-/-} mice, the role of ADAM17 during adult homeostasis as well as the physiological role it plays in regulating the function of other substrates cannot currently be addressed. Further elucidation of the role of ADAM17 in adult mice and in disease models will require the generation of conditional knockout for this multifunctional gene.

8. ADAM19 (Meltrin β , MADDAM)

ADAM19 is a widely expressed and catalytically active ADAM that was first identified in myoblasts along with ADAM12 and ADAM9 (Yagami-Hiromasa *et al.* 1995). While little is currently known about physiologically

relevant substrates, targeted inactivation of ADAM19 has uncovered an essential role in cardiovascular morphogenesis. See also Chapter 9.

8.1 Expression pattern

ADAM19 is ubiquitously expressed in adult tissues, with most prominent expression in the heart, lung, cerebellum, placenta, lymph node, digestive system, leukocytes and in certain cells in the bone (Fritsche *et al.* 2000; Inoue *et al.* 1998; Wei *et al.* 2001). During development, expression of ADAM19 is first seen in the heart and tail bud at 8.0 dpc, and then in the cranial and dorsal root ganglia, and in the ventral horn of the spinal cord (Kurisaki *et al.* 1998). ADAM19 was also identified as a gene specifically expressed in dendritic cells but not in macrophages, indicating that ADAM19 may serve as a marker for the differentiation of dendritic cells from other cells of the myeloid lineage (Fritsche *et al.* 2000; Fritsche *et al.* 2003).

8.2 ADAM19 is implicated in the processing of TRANCE and neuregulin

Although ADAM19 carries a catalytic site consensus sequence for metalloproteases and possesses catalytic activity (Chesneau *et al.* 2003; Wei *et al.* 2001), little is currently known about its substrate profile. To date, only two membrane proteins have been identified as potential substrates of ADAM19, the TNF-family member TRANCE (OPGL, ODF, RANKL), a protein with important roles in osteoclast differentiation, dendritic cell survival and mammary development (Fata *et al.* 2000; Kong *et al.* 2000; Suda *et al.* 1999), and neuregulin, a ligand for ErbB receptors with important roles in development of the heart, nervous system and other organ systems (Chesneau *et al.* 2003; Falls 2003; Shirakabe *et al.* 2001; Wakatsuki *et al.* 2004). It should be noted, however, that there are conflicting results regarding the involvement of ADAM19 in processing neuregulin (discussed below).

8.3 *Adam19*^{-/-} mice have multiple heart defects and most die shortly after birth

ADAM19 gene targeting was done by two independent groups with different strategies (Kurohara *et al.* 2004; Zhou *et al.* 2004). One was performed by using stem cells with a secretory gene trap insertion in ADAM19 (Mitchell *et al.* 2001; Zhou *et al.* 2004) and the other was done by

removal of exons 10 to 12, which contain the catalytic sequence site (Kurohara *et al.* 2004). In both cases, the progeny of matings of heterozygous ADAM19 mutant mice resulted in a Mendelian distribution of the genotype at birth. However about 80% of *Adam19*^{-/-} mice died in the first few days after birth. Histological analysis revealed very similar defects in heart development in both studies, including a membranous ventricular septum defect (VSD) and malformations of aortic, plumononic and tricuspid valves, but not the mitral valve. These heart defects are considered to be the most likely cause of the perinatal lethality in *Adam19*^{-/-} mice. The penetrance of the VSD and aortic and pulmonic valve defects were complete, while the penetrance of the atrioventricular defects (ostium primum atrial septal and tricuspid valve defects) was only partial. This indicates that the proximal portion of the conotruncal endocardial cushion, i.e. the part of the endocardial cushion that gives rise to the ventricular septum and aortic and pulmonic valves, is most severely affected by the removal of ADAM19 activity. In addition to these multiple heart defects, Kurohara *et al.* also pointed out defects in the fasciculation of preganglionic neurons through the adrenal medulla and in muscle development, exemplified by a thinner diaphragm in the absence of ADAM19. The expression pattern of ADAM19 overlaps that of TRANCE in developing bone, and as described above, ADAM19 has been shown to cleave TRANCE *in vitro*. However, no evident major defect in bone development was found in newborn *Adam19*^{-/-} mice (Zhou *et al.* 2004).

The two studies of *Adam19*^{-/-} mice produced conflicting results with respect to the mechanism underlying the defect in heart development, and more specifically, the potential role of ADAM19 in processing of neuregulin. Kurohara *et al.*, showed that PMA-stimulated neuregulin shedding is abolished in *Adam19*^{-/-} fibroblasts *in vitro* when they are *sparsely* plated, but not when they are densely plated. Based on this observation, they hypothesized that the cardiac defects (and also the defects in preganglionic neurons in the adrenal medulla) are caused by aberrant signaling between neuregulin and its ErbB receptors. However, Zhou *et al.* were unable to identify defects in stimulated or constitutive shedding of neuregulin $\beta 1$ and $\beta 2$ in *Adam19*^{-/-} fibroblasts, regardless of the cell density. In addition, Zhou *et al.* demonstrated that overexpression of ADAM19 in Cos7 cells did not increase constitutive or stimulated processing of neuregulin $\beta 1$ and $\beta 2$, even though it did increase shedding of TRANCE, which was used to confirm that ADAM19 was active in Cos7 cells. The reason for this apparent discrepancy remains to be established, but it might be attributed to the differences in experimental design or the materials.

Quite intriguingly, there is a close similarity in the heart phenotype in *Adam17*^{-/-} and *Adam19*^{-/-} mice. A recent study suggests that the heart

defects in *Adam17*^{-/-} mice are caused by aberrant HB-EGF/EGFR signaling (see above, Jackson *et al.* 2003). As for ADAM19, there is no evidence that it participates in the processing and activation of HB-EGF (Zhou *et al.* 2004). Further studies on the roles of ADAMs in activation of ErbB receptors as well as other molecules with a role in heart development will be required to shed new light on the mechanism underlying the role of ADAM19 in heart development.

9. CONCLUSIONS

Evaluation of knockout mice for seven ADAM proteases has uncovered essential roles for ADAMs 10, 17, and 19 in mouse development, and has shown that ADAMs 8, 9, 12 and 15 are not essential for development and adult homeostasis, at least in the controlled and clean environment in which laboratory animals are kept (Table 1). Studies of *Adam10*^{-/-} and *Adam17*^{-/-} mice and cells derived from these animals have revealed that these ADAMs have key regulatory roles in some of the major signaling pathways in cells, including the EGFR and Notch/Delta pathways. Thus ADAM-dependent processing and ectodomain shedding of receptors, ligands and other proteins has emerged as a critical post-translational regulator of the function of at least some substrate proteins. Now that ADAMs have been implicated in a variety of shedding events and the physiological relevance of ectodomain shedding as a post-translational regulator of membrane proteins is well established for proteins such as Notch and some EGFR-ligands, it will be interesting to determine whether ADAM-dependent ectodomain shedding also regulates the function of other substrate proteins. Further studies of knockout mice for ADAMs that are not essential for development (ADAMs 8, 9, 12 and 15), including shedding experiments with cells derived from these animals, and gain of function studies in which these ADAMs that are overexpressed in cells or in transgenic mouse lines will be necessary to help understand what their substrates and functions might be. One important lesson from the published studies of ADAM knockout mice is that the expression pattern can provide important clues as to which tissue a given ADAM might have an important role in. ADAM19, for example, is highly expressed in developing heart valves, and is critical for their proper morphogenesis during development (Zhou *et al.* 2004), while ADAM15 is highly expressed in endothelial cells, and has an important role in pathological neovascularization (Horiuchi *et al.* 2003). Similar challenges that are designed based on the expression pattern of other ADAMs might also reveal unexpected functions of these proteins.

Once a function of an ADAM has been found, it will be important to determine the underlying molecular mechanism. In the case of ADAM15, for example, it will be interesting to test whether the decreased pathological neovascularization in *Adam15*^{-/-} mice can be explained through a defect in shedding molecules with a role in angiogenesis and neovascularization, such as the receptors for the vascular endothelial growth factor (VEGFR1 and 2), and the angiopoietin receptor Tie 2.

Table 1. Ablation of ADAMs in mice and their resulting phenotypes

Gene	Phenotype
ADAM8/MS2	Fertile and viable with no overt phenotype
ADAM9/Meltrin γ	Fertile and viable with no overt phenotype
ADAM10/Kuz	Embryonic lethal at E9.5; multiple defects in several developing organs
ADAM12/Meltrin α	Fertile and viable with no overt phenotype (subtle defects in brown fat-tissue and muscle)
ADAM15/Metarginin	Fertile and viable with no overt phenotype (decreased neovascularization in proliferative retinopathy)
ADAM17/TACE	Perinatally lethal; Multiple defects in several organs
ADAM19/Meltrin β	Perinatally lethal; Multiple heart defects

Finally, in the context of discussing the role of ADAMs in knockout mice it is interesting to note that ADAMs 10 and 17, which are essential for mouse development, have apparent orthologues in *Drosophila melanogaster*, whereas ADAMs 8, 9, 12, 15 and 19 do not. This suggests that the latter group of ADAMs evolved more recently. One possibility is that these ADAMs might therefore have functions in organ systems that have become more highly evolved and specialized in vertebrates, such as the immune system, cardiovascular system (as is the case for ADAMs 15 and 19) or the central nervous system. This further underscores the notion that specific challenges of knockout mice for ADAMs 8, 9, 12, and 15 might reveal roles for these proteins in cells or tissues that are not essential for development, but may instead have important functions in adult animals. It is also possible that the absence of a severe phenotype in mice lacking ADAMs 8, 9, 12 or 15 is due to functional redundancy with other ADAMs, or compensation by other ADAMs. However, mice lacking multiple ADAMs, such as *Adam9/12/15*^{-/-} mice are viable and fertile, and appear normal and healthy (Sahin *et al.* 2004). Thus, to date there is no clear evidence for compensatory or redundant roles of ADAMs that are essential for development, although further studies will be necessary to address this issue more comprehensively. Taken together, the analysis of ADAM knockout mice has provided intriguing insights into the functions of some members of this protein family,

and has established a critical role of ADAMs 10 and 17 as regulatory switches for the Notch and EGFR signaling pathways.

REFERENCES

- Abe, E., Mocharla, H., Yamate, T., Taguchi, Y., Manolagas, S. C., 1999, Meltrin-alpha, a fusion protein involved in multinucleated giant cell and osteoclast formation. *Calcif Tissue Int.* **64**: 508-515.
- Abel, S., Hundhausen, C., Mentlein, R., Schulte, A., Berkhout, T. A., Broadway, N., Hartmann, D., Sedlacek, R., Dietrich, S., Muetze, B., Schuster, B., Kallen, K. J., Saftig, P., Rose-John, S., Ludwig, A., 2004, The transmembrane CXC-chemokine ligand 16 is induced by IFN-gamma and TNF-alpha and shed by the activity of the disintegrin-like metalloproteinase ADAM10. *J Immunol.* **172**: 6362-6372.
- Abram, C. L., Seals, D. F., Pass, I., Salinsky, D., Maurer, L., Roth, T. M., Courtneidge, S. A., 2003, The adaptor protein fish associates with members of the ADAMs family and localizes to podosomes of Src-transformed cells. *J Biol Chem.* **278**: 16844-16851.
- Al-Fakhri, N., Wilhelm, J., Hahn, M., Heidt, M., Hehrlein, F. W., Endisch, A. M., Hupp, T., Cherian, S. M., Bobryshev, Y. V., Lord, R. S., Katz, N., 2003, Increased expression of disintegrin-metalloproteinases ADAM-15 and ADAM-9 following upregulation of integrins alpha5beta1 and alphavbeta3 in atherosclerosis. *J Cell Biochem.* **89**: 808-823.
- Amour, A., Knight, C. G., English, W. R., Webster, A., Slocombe, P. M., Knauper, V., Docherty, A. J., Becherer, J. D., Blobel, C. P., Murphy, G., 2002, The enzymatic activity of ADAM8 and ADAM9 is not regulated by TIMPs. *FEBS Lett.* **524**: 154-158.
- Artavanis-Tsakonas, S., Matsuno, K., Fortini, M. E., 1995, Notch signaling. *Science.* **268**: 225-232.
- Artavanis-Tsakonas, S., Rand, M. D., Lake, R. J., 1999, Notch signaling: cell fate control and signal integration in development. *Science.* **284**: 770-776.
- Asai, M., Hattori, C., Szabo, B., Sasagawa, N., Maruyama, K., Tanuma, S., Ishiura, S., 2003, Putative function of ADAM9, ADAM10, and ADAM17 as APP alpha-secretase. *Biochem Biophys Res Commun.* **301**: 231-235.
- Asakura, M., Kitakaze, M., Takashima, S., Liao, Y., Ishikura, F., Yoshinaka, T., Ohmoto, H., Node, K., Yoshino, K., Ishiguro, H., Asanuma, H., Sanada, S., Matsumura, Y., Takeda, H., Beppu, S., Tada, M., Hori, M., Higashiyama, S., 2002, Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. *Nat Med.* **8**: 35-40.
- Bax, D. V., Messent, A. J., Tart, J., van Hoang, M., Kott, J., Maciewicz, R. A., Humphries, M. J., 2004, Integrin alpha5beta1 and ADAM-17 interact in vitro and co-localize in migrating HeLa cells. *J Biol Chem.* **279**: 22377-22386.
- Bernstein, H. G., Bukowska, A., Krell, D., Bogerts, B., Ansorge, S., Lendeckel, U., 2003, Comparative localization of ADAMs 10 and 15 in human cerebral cortex normal aging, Alzheimer disease and Down syndrome. *J Neurocytol.* **32**: 153-160.
- Black, R. A., Rauch, C. T., Kozlosky, C. J., Peschon, J. J., Slack, J. L., Wolfson, M. F., Castner, B. J., Stocking, K. L., Reddy, P., Srinivasan, S., Nelson, N., Boiani, N., Schooley, K. A., Gerhart, M., Davis, R., Fitzner, J. N., Johnson, R. S., Paxton, R. J., March, C. J., Cerretti, D. P., 1997, A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature.* **385**: 729-733.

- Blobel, C. P., Myles, D. G., Primakoff, P., White, J. M., 1990, Proteolytic processing of a protein involved in sperm-egg fusion correlates with acquisition of fertilization competence. *J Cell Biol.* **111**: 69-78.
- Blobel, C. P. and White, J. M., 1992, Structure, function and evolutionary relationship of proteins containing a disintegrin domain. *Curr Opin Cell Biol.* **4**: 760-765.
- Blobel, C. P., Wolfsberg, T. G., Turck, C. W., Myles, D. G., Primakoff, P., White, J. M., 1992, A potential fusion peptide and an integrin ligand domain in a protein active in sperm-egg fusion. *Nature.* **356**: 248-252.
- Borneman, A., Kuschel, R., Fujisawa-Sehara, A., 2000, Analysis for transcript expression of meltrin alpha in normal, regenerating, and denervated rat muscle. *J Muscle Res Cell Motil.* **21**: 475-480.
- Borrell-Pages, M., Rojo, F., Albanell, J., Baselga, J., Arribas, J., 2003, TACE is required for the activation of the EGFR by TGF-alpha in tumors. *Embo J.* **22**: 1114-1124.
- Bosse, F., Petzold, G., Greiner-Petter, R., Pippirs, U., Gillen, C., Muller, H. W., 2000, Cellular localization of the disintegrin CRII-7/rMDC15 mRNA in rat PNS and CNS and regulated expression in postnatal development and after nerve injury. *Glia.* **32**: 313-327.
- Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J. R., Cumano, A., Roux, P., Black, R. A., Israel, A., 2000, A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. *Mol Cell.* **5**: 207-216.
- Buxbaum, J. D., Liu, K. N., Luo, Y., Slack, J. L., Stocking, K. L., Peschon, J. J., Johnson, R. S., Castner, B. J., Cerretti, D. P., Black, R. A., 1998, Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. *J Biol Chem.* **273**: 27765-27767.
- Cai, H., Kratzschmar, J., Alfandari, D., Hunnicutt, G., Blobel, C. P., 1998, Neural crest-specific and general expression of distinct metalloprotease-disintegrins in early *Xenopus laevis* development. *Dev Biol.* **204**: 508-524.
- Cao, Y., Kang, Q., Zhao, Z., Zolkiewska, A., 2002, Intracellular processing of metalloprotease disintegrin ADAM12. *J Biol Chem.* **277**: 26403-26411.
- Cao, Y., Kang, Q., Zolkiewska, A., 2001, Metalloprotease-disintegrin ADAM 12 interacts with alpha-actinin-1. *Biochem J.* **357**: 353-361.
- Cao, Y., Zhao, Z., Gruszczynska-Biegala, J., Zolkiewska, A., 2003, Role of metalloprotease disintegrin ADAM12 in determination of quiescent reserve cells during myogenic differentiation in vitro. *Mol Cell Biol.* **23**: 6725-6738.
- Chesneau, V., Becherer, J. D., Zheng, Y., Erdjument-Bromage, H., Tempst, P., Blobel, C. P., 2003, Catalytic properties of ADAM19. *J Biol Chem.* **278**: 22331-22340.
- Cho, C., Bunch, D. O., Faure, J. E., Goulding, E. H., Eddy, E. M., Primakoff, P., Myles, D. G., 1998, Fertilization defects in sperm from mice lacking fertilin beta. *Science.* **281**: 1857-1859.
- Choi, S. J., Han, J. H., Roodman, G. D., 2001, ADAM8: a novel osteoclast stimulating factor. *J Bone Miner Res.* **16**: 814-822.
- Condon, T. P., Flournoy, S., Sawyer, G. J., Baker, B. F., Kishimoto, T. K., Bennett, C. F., 2001, ADAM17 but not ADAM10 mediates tumor necrosis factor-alpha and L-selectin shedding from leukocyte membranes. *Antisense Nucleic Acid Drug Dev.* **11**: 107-116.
- Contin, C., Pitard, V., Itai, T., Nagata, S., Moreau, J. F., Dechanet-Merville, J., 2003, Membrane-anchored CD40 is processed by the tumor necrosis factor-alpha-converting enzyme. Implications for CD40 signaling. *J Biol Chem.* **278**: 32801-32809.
- Cruz, A. C., Frank, B. T., Edwards, S. T., Dazin, P. F., Peschon, J. J., Fang, K. C., 2004, Tumor necrosis factor-alpha-converting enzyme controls surface expression of c-Kit and survival of embryonic stem cell-derived mast cells. *J Biol Chem.* **279**: 5612-5620.

- Eliceiri, B. P. and Cheresh, D. A., 2000, Role of α_v integrins during angiogenesis. *Cancer J.* **6**: S245-249.
- Falls, D. L., 2003, Neuregulins: functions, forms, and signaling strategies. *Exp Cell Res.* **284**: 14-30.
- Fambrough, D., Pan, D., Rubin, G. M., Goodman, C. S., 1996, The cell surface metalloprotease/disintegrin Kuzbanian is required for axonal extension in *Drosophila*. *Proc Natl Acad Sci USA.* **93**: 13233-13238.
- Fata, J. E., Kong, Y. Y., Li, J., Sasaki, T., Irie-Sasaki, J., Moorehead, R. A., Elliott, R., Scully, S., Voura, E. B., Lacey, D. L., Boyle, W. J., Khokha, R., Penninger, J. M., 2000, The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. *Cell.* **103**: 41-50.
- Fourie, A. M., Coles, F., Moreno, V., Karlsson, L., 2003, Catalytic activity of ADAM8, ADAM15, and MDC-L (ADAM28) on synthetic peptide substrates and in ectodomain cleavage of CD23. *J Biol Chem.* **278**: 30469-30477.
- Franzke, C. W., Tasanen, K., Borradori, L., Huotari, V., Bruckner-Tuderman, L., 2004, Shedding of Collagen XVII/BP180: STRUCTURAL MOTIFS INFLUENCE CLEAVAGE FROM CELL SURFACE. *J Biol Chem.* **279**: 24521-24529.
- Fritsche, J., Moser, M., Faust, S., Peuker, A., Buttner, R., Andreesen, R., Kreutz, M., 2000, Molecular cloning and characterization of a human metalloprotease disintegrin—a novel marker for dendritic cell differentiation. *Blood.* **96**: 732-739.
- Fritsche, J., Muller, A., Hausmann, M., Rogler, G., Andreesen, R., Kreutz, M., 2003, Inverse regulation of the ADAM-family members, decysin and MADDAM/ADAM19 during monocyte differentiation. *Immunology.* **110**: 450-457.
- Galliano, M. F., Huet, C., Frygeliuss, J., Polgren, A., Wewer, U. M., Engvall, E., 2000, Binding of ADAM12, 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.
- Garton, K. J., Gough, P. J., Blobel, C. P., Murphy, G., Greaves, D. R., Dempsey, P. J., Raines, E. W., 2001, Tumor necrosis factor- α -converting enzyme (ADAM17) mediates the cleavage and shedding of fractalkine (CX3CL1). *J Biol Chem.* **276**: 37993-38001.
- Gilpin, B. J., Loechel, F., Mattei, M. G., Engvall, E., Albrechtsen, R., Wewer, U. M., 1998, A novel, secreted form of human ADAM 12 (meltrin α) provokes myogenesis in vivo. *J Biol Chem.* **273**: 157-166.
- Goddard, D. R., Bunning, R. A., Woodroffe, M. N., 2001, Astrocyte and endothelial cell expression of ADAM 17 (TACE) in adult human CNS. *Glia.* **34**: 267-271.
- Gough, P. J., Garton, K. J., Wille, P. T., Rychlewski, M., Dempsey, P. J., Raines, E. W., 2004, A disintegrin and metalloproteinase 10-mediated cleavage and shedding regulates the cell surface expression of CXC chemokine ligand 16. *J Immunol.* **172**: 3678-3685.
- Greenwald, I., 1998, LIN-12/Notch signaling: lessons from worms and flies. *Genes Dev.* **12**: 1751-1762.
- Grutzmann, R., Foerder, M., Alldinger, I., Staub, E., Brummendorf, T., Ropcke, S., Li, X., Kristiansen, G., Jesnowski, R., Sipos, B., Lohr, M., Luttges, J., Ockert, D., Kloppel, G., Saeger, H. D., Pilarsky, C., 2003, Gene expression profiles of microdissected pancreatic ductal adenocarcinoma. *Virchows Arch.* **443**: 508-517.
- Grutzmann, R., Luttges, J., Sipos, B., Ammerpohl, O., Dobrowolski, F., Alldinger, I., Kersting, S., Ockert, D., Koch, R., Kalthoff, H., Schackert, H. K., Saeger, H. D., Kloppel, G., Pilarsky, C., 2004, ADAM9 expression in pancreatic cancer is associated with tumour type and is a prognostic factor in ductal adenocarcinoma. *Br J Cancer.* **90**: 1053-1058.

- Gschwind, A., Hart, S., Fischer, O. M., Ullrich, A., 2003, TACE cleavage of proamphiregulin regulates GPCR-induced proliferation and motility of cancer cells. *Embo J.* **22**: 2411-2421.
- Hall, R. J. and Erickson, C. A., 2003, ADAM 10: an active metalloprotease expressed during avian epithelial morphogenesis. *Dev Biol.* **256**: 146-159.
- Ham, C., Levkau, B., Raines, E. W., Herren, B., 2002, ADAM15 is an adherens junction molecule whose surface expression can be driven by VE-cadherin. *Exp Cell Res.* **279**: 239-247.
- Harris, H. A., Murrills, R. J., Komm, B. S., 1997, Expression of meltrin-alpha mRNA is not restricted to fusagenic cells. *J Cell Biochem.* **67**: 136-142.
- Harris, R. C., Chung, E., Coffey, R. J., 2003, EGF receptor ligands. *Exp Cell Res.* **284**: 2-13.
- Hart, S., Fischer, O. M., Ullrich, A., 2004, Cannabinoids induce cancer cell proliferation via tumor necrosis factor alpha-converting enzyme (TACE/ADAM17)-mediated transactivation of the epidermal growth factor receptor. *Cancer Res.* **64**: 1943-1950.
- Hartmann, D., de Strooper, B., Serneels, L., Craessaerts, K., Herreman, A., Annaert, W., Umans, L., Lubke, T., Lena Illert, A., von Figura, K., Saftig, P., 2002, The disintegrin/metalloprotease ADAM 10 is essential for Notch signalling but not for alpha-secretase activity in fibroblasts. *Hum Mol Genet.* **11**: 2615-2624.
- Hattori, M., Osterfield, M., Flanagan, J. G., 2000, Regulated cleavage of a contact-mediated axon repellent. *Science.* **289**: 1360-1365.
- Herreman, A., Hartmann, D., Annaert, W., Saftig, P., Craessaerts, K., Serneels, L., Umans, L., Schrijvers, V., Checler, F., Vanderstichele, H., Baekelandt, V., Dressel, R., Cupers, P., Huylebroeck, D., Zwijsen, A., Van Leuven, F., De Strooper, B., 1999, Presenilin 2 deficiency causes a mild pulmonary phenotype and no changes in amyloid precursor protein processing but enhances the embryonic lethal phenotype of presenilin 1 deficiency. *Proc Natl Acad Sci USA.* **96**: 11872-11877.
- Herreman, A., Serneels, L., Annaert, W., Collen, D., Schoonjans, L., De Strooper, B., 2000, Total inactivation of gamma-secretase activity in presenilin-deficient embryonic stem cells. *Nat Cell Biol.* **2**: 461-462.
- Herren, B., Raines, E. W., Ross, R., 1997, Expression of a disintegrin-like protein in cultured human vascular cells and in vivo. *Faseb J.* **11**: 173-180.
- Higuchi, Y., Yasui, A., Matsuura, K., Yamamoto, S., 2002, CD156 transgenic mice. Different responses between inflammatory types. *Pathobiology.* **70**: 47-54.
- Hinkle, C. L., Sunnarborg, S. W., Loiselle, D., Parker, C. E., Stevenson, M., Russell, W. E., Lee, D. C., 2004, Selective roles for tumor necrosis factor alpha-converting enzyme/ADAM17 in the shedding of the epidermal growth factor receptor ligand family: the juxtamembrane stalk determines cleavage efficiency. *J Biol Chem.* **279**: 24179-24188.
- Horiuchi, K., Weskamp, G., Lum, L., Hammes, H. P., Cai, H., Brodie, T. A., Ludwig, T., Chiusaroli, R., Baron, R., Preissner, K. T., Manova, K., Blobel, C. P., 2003, Potential role for ADAM15 in pathological neovascularization in mice. *Mol Cell Biol.* **23**: 5614-5624.
- Howard, L., Lu, X., Mitchell, S., Griffiths, S., Glynn, P., 1996, Molecular cloning of MADM: a catalytically active mammalian disintegrin-metalloprotease expressed in various cell types. *Biochem J.* **317**: 45-50.
- Howard, L., Nelson, K. K., Maciewicz, R. A., Blobel, C. P., 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.
- Howard, L., Zheng, Y., Horrocks, M., Maciewicz, R. A., Blobel, C., 2001, Catalytic activity of ADAM28. *FEBS Lett.* **498**: 82-86.

- Huang, T. F., Holt, J. C., Lukasiewicz, H., Niewiarowski, S., 1987, Trigramin. A low molecular weight peptide inhibiting fibrinogen interaction with platelet receptors expressed on glycoprotein IIb-IIIa complex. *J Biol Chem.* **262**: 16157-16163.
- Huang, X. Z., Wu, J. F., Ferrando, R., Lee, J. H., Wang, Y. L., Farese, R. V., Jr., Sheppard, D., 2000, Fatal bilateral chylothorax in mice lacking the integrin $\alpha 9\beta 1$. *Mol Cell Biol.* **20**: 5208-5215.
- Hundhausen, C., Misztela, D., Berkhout, T. A., Broadway, N., Saftig, P., Reiss, K., Hartmann, D., Fahrenholz, F., Postina, R., Matthews, V., Kallen, K. J., Rose-John, S., Ludwig, A., 2003, The disintegrin-like metalloproteinase ADAM10 is involved in constitutive cleavage of CX3CL1 (fractalkine) and regulates CX3CL1-mediated cell-cell adhesion. *Blood.* **102**: 1186-1195.
- Hussain, I., Hawkins, J., Shikotra, A., Riddell, D. R., Faller, A., Dingwall, C., 2003, Characterization of the ectodomain shedding of the beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1). *J Biol Chem.* **278**: 36264-36268.
- Hynes, R. O., 2002, A reevaluation of integrins as regulators of angiogenesis. *Nat Med.* **8**: 918-921.
- Iba, K., Albrechtsen, R., Gilpin, B., Frohlich, C., Loechel, F., Zolkiewska, A., Ishiguro, K., Kojima, T., Liu, W., Langford, J. K., Sanderson, R. D., Brakebusch, C., Fassler, R., Wewer, U. M., 2000, The cysteine-rich domain of human ADAM 12 supports cell adhesion through syndecans and triggers signaling events that lead to $\beta 1$ integrin-dependent cell spreading. *J Cell Biol.* **149**: 1143-1156.
- Iba, K., Albrechtsen, R., Gilpin, B. J., Loechel, F., Wewer, U. M., 1999, Cysteine-rich domain of human ADAM 12 (meltrin α) supports tumor cell adhesion. *Am J Pathol.* **154**: 1489-1501.
- Inoue, D., Reid, M., Lum, L., Kratzschmar, J., Weskamp, G., Myung, Y. M., Baron, R., Blobel, C. P., 1998, Cloning and initial characterization of mouse meltrin β and analysis of the expression of four metalloprotease-disintegrins in bone cells. *J Biol Chem.* **273**: 4180-4187.
- Ito, N., Nomura, S., Iwase, A., Ito, T., Kikkawa, F., Tsujimoto, M., Ishiura, S., Mizutani, S., 2004, ADAMs, a disintegrin and metalloproteinases, mediate shedding of oxytocinase. *Biochem Biophys Res Commun.* **314**: 1008-1013.
- Iwamoto, R., Yamazaki, S., Asakura, M., Takashima, S., Hasuwa, H., Miyado, K., Adachi, S., Kitakaze, M., Hashimoto, K., Raab, G., Nanba, D., Higashiyama, S., Hori, M., Klagsbrun, M., Mekada, E., 2003, Heparin-binding EGF-like growth factor and ErbB signaling is essential for heart function. *Proc Natl Acad Sci U S A.* **100**: 3221-3226.
- Izumi, Y., Hirata, M., Hasuwa, H., Iwamoto, R., Umata, T., Miyado, K., Tamai, Y., Kurisaki, T., Sehara-Fujisawa, A., Ohno, S., Mekada, E., 1998, A metalloprotease-disintegrin, MDC9/meltrin- γ /ADAM9 and PKC δ are involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor. *Embo J.* **17**: 7260-7272.
- Jackson, L. F., Qiu, T. H., Sunnarborg, S. W., Chang, A., Zhang, C., Patterson, C., Lee, D. C., 2003, Defective valvulogenesis in HB-EGF and TACE-null mice is associated with aberrant BMP signaling. *Embo J.* **22**: 2704-2716.
- Jongeneel, C. V., Bouvier, J., Bairoch, A., 1989, A unique signature identifies a family of zinc-dependent metalloproteases. *FEBS Lett.* **242**: 211-214.
- Kang, Q., Cao, Y., Zolkiewska, A., 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.

- Kang, Q., Cao, Y., Zolkiewska, A., 2001, Direct interaction between the cytoplasmic tail of ADAM 12 and the Src homology 3 domain of p85alpha activates phosphatidylinositol 3-kinase in C2C12 cells. *J Biol Chem.* **276**: 24466-24472.
- Karkkainen, I., Rybnikova, E., Peltto-Huikko, M., Huovila, A. P., 2000, Metalloprotease-disintegrin (ADAM) genes are widely and differentially expressed in the adult CNS. *Mol Cell Neurosci.* **15**: 547-560.
- Kataoka, M., Yoshiyama, K., Matsuura, K., Hijiya, N., Higuchi, Y., Yamamoto, S., 1997, Structure of the murine CD156 gene, characterization of its promoter, and chromosomal location. *J Biol Chem.* **272**: 18209-18215.
- Kawaguchi, N., Xu, X., Tajima, R., Kronqvist, P., Sundberg, C., Loechel, F., Albrechtsen, R., Wewer, U. M., 2002, ADAM 12 protease induces adipogenesis in transgenic mice. *Am J Pathol.* **160**: 1895-1903.
- Kelly, K., Hutchinson, G., Klewe-Nebenius, D., Smith, A., Bartsch, J. W., Horiuchi, K., Manova, K., Docherty, A. J., Blobel, C. P., 2004, Metalloprotease-disintegrin ADAM8: expression pattern analysis and targeted deletion in mice. *Dev Dyn.* (in press).
- Kheradmand, F. and Werb, Z., 2002, Shedding light on sheddases: role in growth and development. *Bioessays.* **24**: 8-12.
- King, N. E., Zimmermann, N., Pope, S. M., Fulkerson, P. C., Nikolaidis, N. M., Mishra, A., Witte, D. P., Rothenberg, M. E., 2004, Expression and regulation of a disintegrin and metalloproteinase (ADAM)8 in experimental asthma. *Am J Respir Cell Mol Biol.* **31**: 257-265.
- Koike, H., Tomioka, S., Sorimachi, H., Saido, T. C., Maruyama, K., Okuyama, A., Fujisawa-Sehara, A., Ohno, S., Suzuki, K., Ishiura, S., 1999, Membrane-anchored metalloprotease MDC9 has an alpha-secretase activity responsible for processing the amyloid precursor protein. *Biochem J.* **343**: 371-375.
- Kojro, E., Gimpl, G., Lammich, S., Marz, W., Fahrenholz, F., 2001, Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha -secretase ADAM 10. *Proc Natl Acad Sci U S A.* **98**: 5815-5820.
- Kong, Y. Y., Boyle, W. J., Penninger, J. M., 2000, Osteoprotegerin ligand: a regulator of immune responses and bone physiology. *Immunol Today.* **21**: 495-502.
- Kratzschmar, J., Lum, L., Blobel, C. P., 1996, Metargidin, a membrane-anchored metalloprotease-disintegrin protein with an RGD integrin binding sequence. *J Biol Chem.* **271**: 4593-4596.
- Krebs, L. T., Xue, Y., Norton, C. R., Shutter, J. R., Maguire, M., Sundberg, J. P., Gallahan, D., Closson, V., Kitajewski, J., Callahan, R., Smith, G. H., Stark, K. L., Gridley, T., 2000, Notch signaling is essential for vascular morphogenesis in mice. *Genes Dev.* **14**: 1343-1352.
- Kronqvist, P., Kawaguchi, N., Albrechtsen, R., Xu, X., Schroder, H. D., Moghadaszadeh, B., Nielsen, F. C., Frohlich, C., Engvall, E., Wewer, U. M., 2002, ADAM12 alleviates the skeletal muscle pathology in mdx dystrophic mice. *Am J Pathol.* **161**: 1535-1540.
- Kurisaki, T., Masuda, A., Osumi, N., Nabeshima, Y., Fujisawa-Sehara, A., 1998, Spatially- and temporally-restricted expression of meltrin alpha (ADAM12) and beta (ADAM19) in mouse embryo. *Mech Dev.* **73**: 211-215.
- Kurisaki, T., Masuda, A., Sudo, K., Sakagami, J., Higashiyama, S., Matsuda, Y., Nagabukuro, A., Tsuji, A., Nabeshima, Y., Asano, M., Iwakura, Y., Sehara-Fujisawa, A., 2003, Phenotypic analysis of Meltrin alpha (ADAM12)-deficient mice: involvement of Meltrin alpha in adipogenesis and myogenesis. *Mol Cell Biol.* **23**: 55-61.

- Kurohara, K., Komatsu, K., Kurisaki, T., Masuda, A., Irie, N., Asano, M., Sudo, K., Nabeshima, Y., Iwakura, Y., Sehara-Fujisawa, A., 2004, Essential roles of Meltrin beta (ADAM19) in heart development. *Dev Biol.* **267**: 14-28.
- Lammich, S., Kojro, E., Postina, R., Gilbert, S., Pfeiffer, R., Jasiewicz, M., Haass, C., Fahrenholz, F., 1999, Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc Natl Acad Sci U S A.* **96**: 3922-3927.
- Le Pabic, H., Bonnier, D., Wewer, U. M., Coutand, A., Musso, O., Baffet, G., Clement, B., Theret, N., 2003, ADAM12 in human liver cancers: TGF-beta-regulated expression in stellate cells is associated with matrix remodeling. *Hepatology.* **37**: 1056-1066.
- Lemjabbar, H. and Basbaum, C., 2002, Platelet-activating factor receptor and ADAM10 mediate responses to Staphylococcus aureus in epithelial cells. *Nat Med.* **8**: 41-46.
- Lieber, T., Kidd, S., Young, M. W., 2002, kuzbanian-mediated cleavage of Drosophila Notch. *Genes Dev.* **16**: 209-221.
- Loechel, F., Fox, J. W., Murphy, G., Albrechtsen, R., Wewer, U. M., 2000, ADAM 12-S cleaves IGFBP-3 and IGFBP-5 and is inhibited by TIMP-3. *Biochem Biophys Res Commun.* **278**: 511-515.
- Loechel, F., Gilpin, B. J., Engvall, E., Albrechtsen, R., Wewer, U. M., 1998, Human ADAM 12 (meltrin alpha) is an active metalloprotease. *J Biol Chem.* **273**: 16993-16997.
- Lopez-Perez, E., Zhang, Y., Frank, S. J., Creemers, J., Seidah, N., Checler, F., 2001, Constitutive alpha-secretase cleavage of the beta-amyloid precursor protein in the furin-deficient LoVo cell line: involvement of the pro-hormone convertase 7 and the disintegrin metalloprotease ADAM10. *J Neurochem.* **76**: 1532-1539.
- Lum, L., Reid, M. S., Blobel, C. P., 1998, Intracellular maturation of the mouse metalloprotease disintegrin MDC15. *J Biol Chem.* **273**: 26236-26247.
- Mandelin, J., Li, T. F., Hukkanen, M. V., Liljestrom, M., Chen, Z. K., Santavirta, S., Kitti, U., Konttinen, Y. T., 2003, Increased expression of a novel osteoclast-stimulating factor, ADAM8, in interface tissue around loosened hip prostheses. *J Rheumatol.* **30**: 2033-2038.
- Marino, M. W., Dunn, A., Grail, D., Inglese, M., Noguchi, Y., Richards, E., Jungbluth, A., Wada, H., Moore, M., Williamson, B., Basu, S., Old, L. J., 1997, Characterization of tumor necrosis factor-deficient mice. *Proc Natl Acad Sci U S A.* **94**: 8093-8098.
- Martin, J., Eynstone, L. V., Davies, M., Williams, J. D., Steadman, R., 2002, The role of ADAM 15 in glomerular mesangial cell migration. *J Biol Chem.* **277**: 33683-33689.
- Matthews, V., Schuster, B., Schutze, S., Bussmeyer, I., Ludwig, A., Hundhausen, C., Sadowski, T., Saftig, P., Hartmann, D., Kallen, K. J., Rose-John, S., 2003, Cellular cholesterol depletion triggers shedding of the human interleukin-6 receptor by ADAM10 and ADAM17 (TACE). *J Biol Chem.* **278**: 38829-38839.
- Mechtersheimer, S., Gutwein, P., Agmon-Levin, N., Stoeck, A., Oleszewski, M., Riedle, S., Postina, R., Fahrenholz, F., Fogel, M., Lemmon, V., Altevogt, P., 2001, Ectodomain shedding of L1 adhesion molecule promotes cell migration by autocrine binding to integrins. *J Cell Biol.* **155**: 661-673.
- Millichip, M. I., Dallas, D. J., Wu, E., Dale, S., McKie, N., 1998, The metallo-disintegrin ADAM10 (MAD10) from bovine kidney has type IV collagenase activity in vitro. *Biochem Biophys Res Commun.* **245**: 594-598.
- Mitchell, K. J., Pinson, K. I., Kelly, O. G., Brennan, J., Zupicich, J., Scherz, P., Leighton, P. A., Goodrich, L. V., Lu, X., Avery, B. J., Tate, P., Dill, K., Pangilinan, E., Wakenight, P., Tessier-Lavigne, M., Skarnes, W. C., 2001, Functional analysis of secreted and transmembrane proteins critical to mouse development. *Nat Genet.* **28**: 241-249.

- Mori, S., Tanaka, M., Nanba, D., Nishiwaki, E., Ishiguro, H., Higashiyama, S., Matsuura, N., 2003, PACSIN3 binds ADAM12/meltrin alpha and up-regulates ectodomain shedding of heparin-binding epidermal growth factor-like growth factor. *J Biol Chem.* **278**: 46029-46034.
- Moss, M. L., Jin, S. L., Milla, M. E., Bickett, D. M., Burkhart, W., Carter, H. L., Chen, W. J., Clay, W. C., Didsbury, J. R., Hassler, D., Hoffman, C. R., Kost, T. A., Lambert, M. H., Leesnitzer, M. A., McCauley, P., McGeehan, G., Mitchell, J., Moyer, M., Pahel, G., Rocque, W., Overton, L. K., Schoenen, F., Seaton, T., Su, J. L., Becherer, J. D., *et al.*, 1997, Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. *Nature.* **385**: 733-736.
- Mumm, J. S., Schroeter, E. H., Saxena, M. T., Griesemer, A., Tian, X., Pan, D. J., Ray, W. J., Kopan, R., 2000, A ligand-induced extracellular cleavage regulates gamma-secretase-like proteolytic activation of Notch1. *Mol Cell.* **5**: 197-206.
- Murai, T., Miyazaki, Y., Nishinakamura, H., Sugahara, K. N., Miyauchi, T., Sako, Y., Yanagida, T., Miyasaka, M., 2004, Engagement of CD44 promotes Rac activation and CD44 cleavage during tumor cell migration. *J Biol Chem.* **279**: 4541-4550.
- Nath, D., Slocombe, P. M., Stephens, P. E., Warn, A., Hutchinson, G. R., Yamada, K. M., Docherty, A. J., Murphy, G., 1999, Interaction of metargidin (ADAM-15) with alphavbeta3 and alpha5beta1 integrins on different haemopoietic cells. *J Cell Sci.* **112**: 579-587.
- Nath, D., Slocombe, P. M., Webster, A., Stephens, P. E., Docherty, A. J., Murphy, G., 2000, Meltrin gamma(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.
- Naus, S., Richter, M., Wildeboer, D., Schachner, M., Moss, M., Bartsch, J. W., 2004, Ectodomain shedding of the neural recognition molecule CHL1 by the metalloprotease-disintegrin ADAM8 promotes neurite outgrowth and suppresses neuronal cell death. *J Biol Chem.*
- Navarro, V., Vincent, J. P., Mazella, J., 2002, Shedding of the luminal domain of the neurotensin receptor-3/sortilin in the HT29 cell line. *Biochem Biophys Res Commun.* **298**: 760-764.
- Nelson, K. K., Schlondorff, J., Blobel, C. P., 1999, Evidence for an interaction of the metalloprotease-disintegrin tumour necrosis factor alpha convertase (TACE) with mitotic arrest deficient 2 (MAD2), and of the metalloprotease-disintegrin MDC9 with a novel MAD2-related protein, MAD2beta. *Biochem J.* **343**: 673-680.
- Newton, R. C., Solomon, K. A., Covington, M. B., Decicco, C. P., Haley, P. J., Friedman, S. M., Vaddi, K., 2001, Biology of TACE inhibition. *Ann Rheum Dis.* **60**: iii25-32.
- Niewiarowski, S., McLane, M. A., Kloczewiak, M., Stewart, G. J., 1994, Disintegrins and other naturally occurring antagonists of platelet fibrinogen receptors. *Semin Hematol.* **31**: 289-300.
- Ohta, S., Harigai, M., Tanaka, M., Kawaguchi, Y., Sugiura, T., Takagi, K., Fukasawa, C., Hara, M., Kamatani, N., 2001, Tumor necrosis factor-alpha (TNF-alpha) converting enzyme contributes to production of TNF-alpha in synovial tissues from patients with rheumatoid arthritis. *J Rheumatol.* **28**: 1756-1763.
- O'Shea, C., McKie, N., Buggy, Y., Duggan, C., Hill, A. D., McDermott, E., O'Higgins, N., Duffy, M. J., 2003, Expression of ADAM-9 mRNA and protein in human breast cancer. *Int J Cancer.* **105**: 754-761.
- 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.

- Pasparakis, M., Alexopoulou, L., Episkopou, V., Kollias, G., 1996, Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med.* **184**: 1397-1411.
- Patel, I. R., Attur, M. G., Patel, R. N., Stuchin, S. A., Abagyan, R. A., Abramson, S. B., Amin, A. R., 1998, TNF-alpha convertase enzyme from human arthritis-affected cartilage: isolation of cDNA by differential display, expression of the active enzyme, and regulation of TNF-alpha. *J Immunol.* **160**: 4570-4579.
- Peiretti, F., Deprez-Beauclair, P., Bonardo, B., Aubert, H., Juhan-Vague, I., Nalbone, G., 2003, Identification of SAP97 as an intracellular binding partner of TACE. *J Cell Sci.* **116**: 1949-1957.
- Peschon, J. J., Slack, J. L., Reddy, P., Stocking, K. L., Sunnarborg, S. W., Lee, D. C., Russell, W. E., Castner, B. J., Johnson, R. S., Fitzner, J. N., Boyce, R. W., Nelson, N., Kozlosky, C. J., Wolfson, M. F., Rauch, C. T., Cerretti, D. P., Paxton, R. J., March, C. J., Black, R. A., 1998a, An essential role for ectodomain shedding in mammalian development. *Science.* **282**: 1281-1284.
- Peschon, J. J., Torrance, D. S., Stocking, K. L., Glaccum, M. B., Otten, C., Willis, C. R., Charrier, K., Morrissey, P. J., Ware, C. B., Mohler, K. M., 1998b, TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. *J Immunol.* **160**: 943-952.
- Poghosyan, Z., Robbins, S. M., Houslay, M. D., Webster, A., Murphy, G., Edwards, D. R., 2002, Phosphorylation-dependent interactions between ADAM15 cytoplasmic domain and Src family protein-tyrosine kinases. *J Biol Chem.* **277**: 4999-5007.
- Postina, R., Schroeder, A., Dewachter, I., Bohl, J., Schmitt, U., Kojro, E., Prinzen, C., Endres, K., Hiemke, C., Blessing, M., Flamez, P., Dequenue, A., Godaux, E., Van Leuven, F., Fahrenholz, F., 2004, A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. *J Clin Invest.* **113**: 1456-1464.
- Prenzel, N., Zwick, E., Daub, H., Leserer, M., Abraham, R., Wallasch, C., Ullrich, A., 1999, EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature.* **402**: 884-888.
- Primakoff, P., Hyatt, H., Tredick-Kline, J., 1987, Identification and purification of a sperm surface protein with a potential role in sperm-egg membrane fusion. *J Cell Biol.* **104**: 141-149.
- Primakoff, P. and Myles, D. G., 2000, The ADAM gene family: surface proteins with adhesion and protease activity. *Trends Genet.* **16**: 83-87.
- Primakoff, P. and Myles, D. G., 2002, Penetration, adhesion, and fusion in mammalian sperm-egg interaction. *Science.* **296**: 2183-2185.
- Rio, C., Buxbaum, J. D., Peschon, J. J., Corfas, G., 2000, Tumor necrosis factor-alpha-converting enzyme is required for cleavage of erbB4/HER4. *J Biol Chem.* **275**: 10379-10387.
- Roghani, M., Becherer, J. D., Moss, M. L., Atherton, R. E., Erdjument-Bromage, H., Arribas, J., Blackburn, R. K., Weskamp, G., Tempst, P., Blobel, C. P., 1999, Metalloprotease-disintegrin MDC9: intracellular maturation and catalytic activity. *J Biol Chem.* **274**: 3531-3540.
- Rooke, J., Pan, D., Xu, T., Rubin, G. M., 1996, KUZ, a conserved metalloprotease-disintegrin protein with two roles in Drosophila neurogenesis. *Science.* **273**: 1227-1231.

- Sahin, U., Weskamp, G., Kelly, K., Zhou, H. M., Higashiyama, S., Peschon, J., Hartmann, D., Saftig, P., Blobel, C. P., 2004, Distinct roles for ADAM10 and ADAM17 in ectodomain shedding of six EGFR ligands. *J Cell Biol.* **164**: 769-779.
- Schafer, B., Gschwind, A., Ullrich, A., 2004, Multiple G-protein-coupled receptor signals converge on the epidermal growth factor receptor to promote migration and invasion. *Oncogene.* **23**: 991-999.
- Schlomann, U., Rathke-Hartlieb, S., Yamamoto, S., Jockusch, H., Bartsch, J. W., 2000, Tumor necrosis factor alpha induces a metalloprotease-disintegrin, ADAM8 (CD 156): implications for neuron-glia interactions during neurodegeneration. *J Neurosci.* **20**: 7964-7971.
- Schlomann, U., Wildeboer, D., Webster, A., Antropova, O., Zeuschner, D., Knight, C. G., Docherty, A. J., Lambert, M., Skelton, L., Jockusch, H., Bartsch, J. W., 2002, The metalloprotease disintegrin ADAM8. Processing by autocatalysis is required for proteolytic activity and cell adhesion. *J Biol Chem.* **277**: 48210-48219.
- 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.
- Seals, D. F. and Courtneidge, S. A., 2003, The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev.* **17**: 7-30.
- Selkoe, D. J. and Schenk, D., 2003, Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. *Annu Rev Pharmacol Toxicol.* **43**: 545-584.
- Setoguchi, M., Nasu, N., Yoshida, S., Higuchi, Y., Akizuki, S., Yamamoto, S., 1989, Mouse and human CD14 (myeloid cell-specific leucine-rich glycoprotein) primary structure deduced from cDNA clones. *Biochim Biophys Acta.* **1008**: 213-222.
- Shi, W., Chen, H., Sun, J., Buckley, S., Zhao, J., Anderson, K. D., Williams, R. G., Warburton, D., 2003, TACE is required for fetal murine cardiac development and modeling. *Dev Biol.* **261**: 371-380.
- Shi, Z., Xu, W., Loechel, F., Wewer, U. M., Murphy, L. J., 2000, ADAM 12, a disintegrin metalloprotease, interacts with insulin-like growth factor-binding protein-3. *J Biol Chem.* **275**: 18574-18580.
- Shintani, Y., Higashiyama, S., Ohta, M., Hirabayashi, H., Yamamoto, S., Yoshimasu, T., Matsuda, H., Matsuura, N., 2004, Overexpression of ADAM9 in Non-Small Cell Lung Cancer Correlates with Brain Metastasis. *Cancer Res.* **64**: 4190-4196.
- Shirakabe, K., Wakatsuki, S., Kurisaki, T., Fujisawa-Sehara, A., 2001, Roles of Meltrin beta /ADAM19 in the processing of neuregulin. *J Biol Chem.* **276**: 9352-9358.
- Slack, B. E., Ma, L. K., Seah, C. C., 2001, Constitutive shedding of the amyloid precursor protein ectodomain is up-regulated by tumour necrosis factor-alpha converting enzyme. *Biochem J.* **357**: 787-794.
- Stocker, W., Grams, F., Baumann, U., Reinemer, P., Gomis-Ruth, F. X., McKay, D. B., Bode, W., 1995, The metzincins--topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases. *Protein Sci.* **4**: 823-840.
- Suda, T., Takahashi, N., Udagawa, N., Jimi, E., Gillespie, M. T., Martin, T. J., 1999, Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocr Rev.* **20**: 345-357.
- Sunnarborg, S. W., Hinkle, C. L., Stevenson, M., Russell, W. E., Raska, C. S., Peschon, J. J., Castner, B. J., Gerhart, M. J., Paxton, R. J., Black, R. A., Lee, D. C., 2002, Tumor necrosis factor-alpha converting enzyme (TACE) regulates epidermal growth factor receptor ligand availability. *J Biol Chem.* **277**: 12838-12845.

- Suzuki, A., Kadota, N., Hara, T., Nakagami, Y., Izumi, T., Takenawa, T., Sabe, H., Endo, T., 2000, Meltrin alpha cytoplasmic domain interacts with SH3 domains of Src and Grb2 and is phosphorylated by v-Src. *Oncogene*. **19**: 5842-5850.
- Tannapfel, A., Anhalt, K., Hausermann, P., Sommerer, F., Benicke, M., Uhlmann, D., Witzigmann, H., Hauss, J., Wittekind, C., 2003, Identification of novel proteins associated with hepatocellular carcinomas using protein microarrays. *J Pathol*. **201**: 238-249.
- Thathiah, A., Blobel, C. P., Carson, D. D., 2003, Tumor necrosis factor-alpha converting enzyme/ADAM 17 mediates MUC1 shedding. *J Biol Chem*. **278**: 3386-3394.
- Thodeti, C. K., Albrechtsen, R., Grauslund, M., Asmar, M., Larsson, C., Takada, Y., Mercurio, A. M., Couchman, J. R., Wewer, U. M., 2003, ADAM12/syndecan-4 signaling promotes beta 1 integrin-dependent cell spreading through protein kinase Calpha and RhoA. *J Biol Chem*. **278**: 9576-9584.
- Trochon-Joseph, V., Martel-Renoir, D., Mir, L. M., Thomaidis, A., Opolon, P., Connault, E., Li, H., Grenet, C., Fauvel-Lafeve, F., Soria, J., Legrand, C., Soria, C., Perricaudet, M., Lu, H., 2004, Evidence of antiangiogenic and antimetastatic activities of the recombinant disintegrin domain of metargidin. *Cancer Res*. **64**: 2062-2069.
- Vincent, B., Paitel, E., Saftig, P., Frobert, Y., Hartmann, D., De Strooper, B., Grassi, J., Lopez-Perez, E., Checler, F., 2001, The disintegrins ADAM10 and TACE contribute to the constitutive and phorbol ester-regulated normal cleavage of the cellular prion protein. *J Biol Chem*. **276**: 37743-37746.
- Wakatsuki, S., Kurisaki, T., Sehara-Fujisawa, A., 2004, Lipid rafts identified as locations of ectodomain shedding mediated by Meltrin beta/ADAM19. *J Neurochem*. **89**: 119-123.
- Wei, P., Zhao, Y. G., Zhuang, L., Ruben, S., Sang, Q. X., 2001, Expression and enzymatic activity of human disintegrin and metalloproteinase ADAM19/meltrin beta. *Biochem Biophys Res Commun*. **280**: 744-755.
- Weskamp, G. and Blobel, C. P., 1994, A family of cellular proteins related to snake venom disintegrins. *Proc Natl Acad Sci U S A*. **91**: 2748-2751.
- Weskamp, G., Cai, H., Brodie, T. A., Higashiyama, S., Manova, K., Ludwig, T., Blobel, C. P., 2002, Mice lacking the metalloprotease-disintegrin MDC9 (ADAM9) have no evident major abnormalities during development or adult life. *Mol Cell Biol*. **22**: 1537-1544.
- Weskamp, G., Kratzschmar, J., Reid, M. S., Blobel, C. P., 1996, MDC9, a widely expressed cellular disintegrin containing cytoplasmic SH3 ligand domains. *J Cell Biol*. **132**: 717-726.
- Weskamp, G., Schlondorff, J., Lum, L., Becherer, J. D., Kim, T. W., Saftig, P., Hartmann, D., Murphy, G., Blobel, C. P., 2004, Evidence for a critical role of the tumor necrosis factor alpha convertase (TACE) in ectodomain shedding of the p75 neurotrophin receptor (p75NTR). *J Biol Chem*. **279**: 4241-4249.
- White, J. M., 2003, ADAMs: modulators of cell-cell and cell-matrix interactions. *Curr Opin Cell Biol*. **15**: 598-606.
- Wolfsberg, T. G., Bazan, J. F., Blobel, C. P., Myles, D. G., Primakoff, P., White, J. M., 1993, The precursor region of a protein active in sperm-egg fusion contains a metalloprotease and a disintegrin domain: structural, functional, and evolutionary implications. *Proc Natl Acad Sci U S A*. **90**: 10783-10787.
- Wolfsberg, T. G., Primakoff, P., Myles, D. G., White, J. M., 1995a, 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.
- Wolfsberg, T. G., Straight, P. D., Gerena, R. L., Huovila, A. P., Primakoff, P., Myles, D. G., White, J. M., 1995b, ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. *Dev Biol*. **169**: 378-383.

- Yagami-Hiromasa, T., Sato, T., Kurisaki, T., Kamijo, K., Nabeshima, Y., Fujisawa-Sehara, A., 1995, A metalloprotease-disintegrin participating in myoblast fusion. *Nature*. **377**: 652-656.
- Yan, Y., Shirakabe, K., Werb, Z., 2002, The metalloprotease Kuzbanian (ADAM10) mediates the transactivation of EGF receptor by G protein-coupled receptors. *J Cell Biol*. **158**: 221-226.
- Yoshida, S., Setoguchi, M., Higuchi, Y., Akizuki, S., Yamamoto, S., 1990, Molecular cloning of cDNA encoding MS2 antigen, a novel cell surface antigen strongly expressed in murine monocytic lineage. *Int Immunol*. **2**: 585-591.
- Yoshiyama, K., Higuchi, Y., Kataoka, M., Matsuura, K., Yamamoto, S., 1997, CD156 (human ADAM8): expression, primary amino acid sequence, and gene location. *Genomics*. **41**: 56-62.
- Zhang, X. P., Kamata, T., Yokoyama, K., Puzon-McLaughlin, W., Takada, Y., 1998, Specific interaction of the recombinant disintegrin-like domain of MDC-15 (metargidin, ADAM-15) with integrin α v β 3. *J Biol Chem*. **273**: 7345-7350.
- Zhang, Y., Jiang, J., Black, R. A., Baumann, G., Frank, S. J., 2000, Tumor necrosis factor- α converting enzyme (TACE) is a growth hormone binding protein (GHBP) sheddase: the metalloprotease TACE/ADAM-17 is critical for (PMA-induced) GH receptor proteolysis and GHBP generation. *Endocrinology*. **141**: 4342-4348.
- Zhao, J., Chen, H., Peschon, J. J., Shi, W., Zhang, Y., Frank, S. J., Warburton, D., 2001, Pulmonary hypoplasia in mice lacking tumor necrosis factor- α converting enzyme indicates an indispensable role for cell surface protein shedding during embryonic lung branching morphogenesis. *Dev Biol*. **232**: 204-218.
- Zheng, Y., Schlondorff, J., Blobel, C. P., 2002, Evidence for regulation of the tumor necrosis factor α -convertase (TACE) by protein-tyrosine phosphatase PTPH1. *J Biol Chem*. **277**: 42463-42470.
- Zhou, H. M., Weskamp, G., Chesneau, V., Sahin, U., Vortkamp, A., Horiuchi, K., Chiusaroli, R., Hahn, R., Wilkes, D., Fisher, P., Baron, R., Manova, K., Basson, C. T., Hempstead, B., Blobel, C. P., 2004, Essential role for ADAM19 in cardiovascular morphogenesis. *Mol Cell Biol*. **24**: 96-104.
- Zhou, M., Graham, R., Russell, G., Croucher, P. I., 2001, MDC-9 (ADAM-9/Meltrin gamma) functions as an adhesion molecule by binding the α (v) β (5) integrin. *Biochem Biophys Res Commun*. **280**: 574-580.
- Zou, J., Zhu, F., Liu, J., Wang, W., Zhang, R., Garlisi, C. G., Liu, Y. H., Wang, S., Shah, H., Wan, Y., Umland, S. P., 2004, Catalytic Activity of Human ADAM33. *J Biol Chem*. **279**: 9818-9830.



<http://www.springer.com/978-0-387-25149-3>

The ADAM Family of Proteases

Hooper, N.M.; Lendeckel, U. (Eds.)

2005, XIV, 344 p., Hardcover

ISBN: 978-0-387-25149-3