Chapter 2

STUDIES FROM ADAM KNOCKOUT MICE

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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 TNFα, 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 TNFα 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 TNFα (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 α1-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-glial 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 αvβ5 and α6β1 (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, MDAM)**

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 Aβ 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 Aβ 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 endogeneous 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
Studied from Adam knockout mice (Herrema 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 (Herrema et al. 1999; Herrema 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, $\alpha$-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 $\alpha$-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 $\alpha$)

ADAM12 was initially identified along with ADAM19 (meltrin $\beta$) and ADAM9 (meltrin $\gamma$) 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 periostium 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),
oxtocinase (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 α9β1 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 metarginidin 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 αβ3 and αβ1 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 an anti-oncogenic activity through suppressing tumor angiogenesis (Trochon-Joseph et al. 2004). Both mouse and human ADAM15 have been shown to interact with α9β1 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), TGFα 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 TGFα 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, α9β1 integrin can interact with several ADAMs including ADAM9, 12 and 15. Mice lacking α9β1 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 α9β1 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 TNFα (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 TNFα converting enzyme (TACE), i.e. the enzyme that releases TNFα 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 TNFα, 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).
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 α5β1 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-/- Tgfα-/- 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 TNFα 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 TNFα (at the time, TNFα was the only known substrate for ADAM17), this severe phenotype was quite unexpected because mice lacking TNFα 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 TGFα. 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 TGFα, 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 TNFα.

Consistent with this notion, the processing of TGFα 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, plumonic 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 atroventricular 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 β1 and β2 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 β1 and β2, 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 (VEFGR1 and 2), and the angiopoietin receptor Tie 2.

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

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

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


Hoiruchi and Blobel


STUDIES FROM ADAM KNOCKOUT MICE


