The FEBS Journal

FEBS PRESS science publishing by scientists

STATE-OF-THE-ART REVIEW

The protease ADAM17 at the crossroads of disease: revisiting its significance in inflammation, cancer, and beyond

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Keywords
ADAM17; cancer; cardiovascular diseases; ectodomain shedding; inflammation

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(Received 14 June 2023, revised 4 July 2023, accepted 2 August 2023)

doi:10.1111/febs.16923

The protease A Disintegrin And Metalloproteinase 17 (ADAM17) plays a central role in the pathophysiology of several diseases. ADAM17 is involved in the cleavage and shedding of at least 80 known membrane-tethered proteins, which subsequently modulate several intracellular signaling pathways, and therefore alter cell behavior. Dysregulated expression and/or activation of ADAM17 has been linked to a wide range of autoimmune and inflammatory diseases, cancer, and cardiovascular disease. In this review, we provide an overview of the current state of knowledge from preclinical models and clinical data on the diverse pathophysiological roles of ADAM17, and discuss the mechanisms underlying ADAM17-mediated protein shedding and the potential therapeutic implications of targeting ADAM17 in these diseases.

Introduction

Proteases are enzymes that catalyze the proteolytic hydrolysis of peptide bonds, resulting in either the release of active peptides or the degradation of proteins. These proteolytic events control a diverse array of biological processes in all living cells, including cell proliferation and differentiation, cell cycle, tissue

Abbreviations
A17pro, ADAM17 prodomain; ACE2, angiotensin-converting enzyme type 2; ADAM17, A Disintegrin And Metalloproteinase 17; ADAMs, A Disintegrin And Metalloproteinases; ADAMTS, ADAM with thrombospondin motifs; CIA, collagen induced arthritis; CLP, caecal ligation and puncture; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CRC, colorectal cancer; DOCA, deoxycorticosterone acetate; DSS, dextran sulfate sodium; EGFR, epidermal growth factor receptor; ERK1/2, extracellular signal regulated protein kinase; HF, heart failure; HFD, High-fat diet; HNSCC, head and neck squamous cell carcinoma; IL-6, interleukin-6; IL-6R, interleukin-6 receptor; iRhoms, rhomboid family of intramembrane pseudoproteases; iTAP, iRhom tail-associated protein; JAK/STAT, Janus kinase/signal transducer and activator of transcription; LPS, lipopolysaccharides; MAPKs, mitogen-activated protein kinases; MI, myocardial infarction; MMPs, matrix metalloproteinases; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NNK, nicotine-derived nitrosamine ketone; NSCLC, non-small cell lung cancer; PDX, patient-derived xenograft; PKB, protein kinase B; PKCα, protein kinase Cα; PMA, phorbol-12-myristate-13-acetate; shRNA, short hairpin RNA; sIL-6R, soluble form of interleukin-6 receptor; TACE, TNFα converting enzyme; TAILS, terminal amine isotopic labeling of substrates; TAPI, TNFα protease inhibitor; TGFα, transforming growth factor α; Thr735, threonine 735 residue; TIMP3, tissue inhibitor of metalloproteinases-3; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNFR1, tumor necrosis factor receptor 1; TNFα, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule 1; VEGFR2, vascular endothelial growth factor receptor 2; WT, wild-type.

morphogenesis and remodeling, angiogenesis, fertilization, hemostasis, inflammation, immunity, autophagy, senescence, and cell death [1,2]. In humans, there are almost 600 proteases identified, which can be classified based on their different mechanism of proteolysis into five distinct classes: aspartic, metallo, cysteine, serine, and threonine proteases [1,2]. Consistent with their diverse roles in cellular physiology, the biological actions of proteases are strictly controlled transcriptionally and post-transcriptionally in the context of complex networks comprising proteases, substrates, cofactors, inhibitors, adaptors, receptors, and binding proteins, and imbalances in their activities have been demonstrated to be critical in the development of a myriad of pathologies, such as inflammation and cancer [1,2].

Metalloproteinases are enzymes that require a metal ion, mainly zinc, for their catalytic activity, which include matrix metalloproteinases (MMPs), A Disintegrin And Metalloproteinases (ADAMs), and ADAM with thrombospondin motifs (ADAMTS) [3,4]. The ADAMs are a family of transmembrane and secreted proteins of approximately 750 amino acids in length [4]. In humans, there are 21 functional ADAMs, 13 of which possess the characteristic zinc-binding active site (HEXGHXXGXXHD) in their metalloproteinase domain that is indicative of their proteolytic activity [5,6]. ADAM10 and ADAM17 are the most studied ADAMs, and being closely related it is not surprising that among their collective repertoire of over 100 substrates, many are shared between these proteases [6,7]. A defining feature of these ADAMs is their orchestration of the irreversible shedding of the extracellular domain of membrane-tethered proteins as biologically active soluble signals that can act on a plethora of cell types in various modes, namely paracrine, autocrine, and juxtacrine (Fig. 1). Indeed, this quantitative and qualitative diversity in the signaling capabilities of these ADAMs in many biological systems of the body has underpinned the prominent pathophysiological roles assigned thus far to ADAM10 and ADAM17 [7,8]. In this respect, in recent years, ADAM17 in particular has attracted considerable interest as a driver of autoimmune diseases, acute and chronic inflammation, and cancer, which has largely stemmed from studies employing the coupling of genetic and/or inhibitor-based targeting strategies against ADAM17 in preclinical disease mouse models [7,9–17]. This has led to increasing speculation that the treatment of many such disease states with drugs that block the actions of ADAM17 may present novel therapeutic avenues in the clinic. In this review, we provide an update on the complex regulation and biology of the ADAM17 protease, and discuss the clinical potential of ADAM17 and its substrates as therapeutic targets and biomarkers in numerous disease states.

The protease A Disintegrin And Metalloproteinase 17 (ADAM17)

The protease ADAM17, also known as tumor necrosis factor α (TNFα)-converting enzyme (TACE), is a type I transmembrane cell-surface metalloprotease that is widely expressed in various tissues and cell types [7,10,18–20]. The ADAM17 protein consists of a prodomain, a catalytic domain, a disintegrin domain, a cysteine-rich membrane proximal domain, a single transmembrane domain and an intracellular cytoplasmic domain (Fig. 1) [21]. The prodomain of ADAM17 (A17pro) acts as a chaperone and endogenous inhibitor for ADAM17, which requires cleavage by furin proteases to release its mature/active form [10,21]. The protease activity of ADAM17 is tightly regulated through various mechanisms, including post-translational modification such as phosphorylation, conformational changes, as well as interaction with a myriad of interacting partners and cellular signaling molecules [7]. For instance, the rhomboid family of intramembrane pseudoproteases (iRhoms; iRhom1, gene name *RHBDF1* and iRhom2, gene name *RHBDF2*) have gained considerable attention recently due to their emergence as key regulators of the maturation, stability, plasma membrane trafficking, activation, and substrate specificity of ADAM17 [22–28]. The absolute functional requirement by ADAM17 for iRhoms was demonstrated in $Rhbdf1^{-/-}/Rhbdf2^{-/-}$ mice, in which ADAM17 maturation and trafficking, and proteolytic shedding of its substrates, was abolished [23,29]. Mechanistically, the stimulation of ADAM17 has been shown to induce mitogen-activated protein kinases (MAPKs)-dependent phosphorylation of the N-terminal cytoplasmic tail of iRhom2, which then recruits 14–3-3 proteins, culminating in the dissociation of ADAM17 transmembrane domain/iRhom2 complex and enhancing ADAM17 shedding activity [30].

The protein–protein interactions and phosphorylation events on the cytoplasmic domain of ADAM17 have also been suggested to play important roles in regulating its function. Truncation of the ADAM17 cytoplasmic domain in mice resulted in viable mice with a mild ADAM17 hypomorphic phenotype in all tissues due to reduced shedding of epidermal growth factor receptor (EGFR) ligands, reminiscent of ADAM17 deficiency in mice [31]. However, truncated ADAM17 was shown to respond to its exogenous post-translational activator phorbol-12-myristate-13-acetate (PMA), suggesting that the cytoplasmic

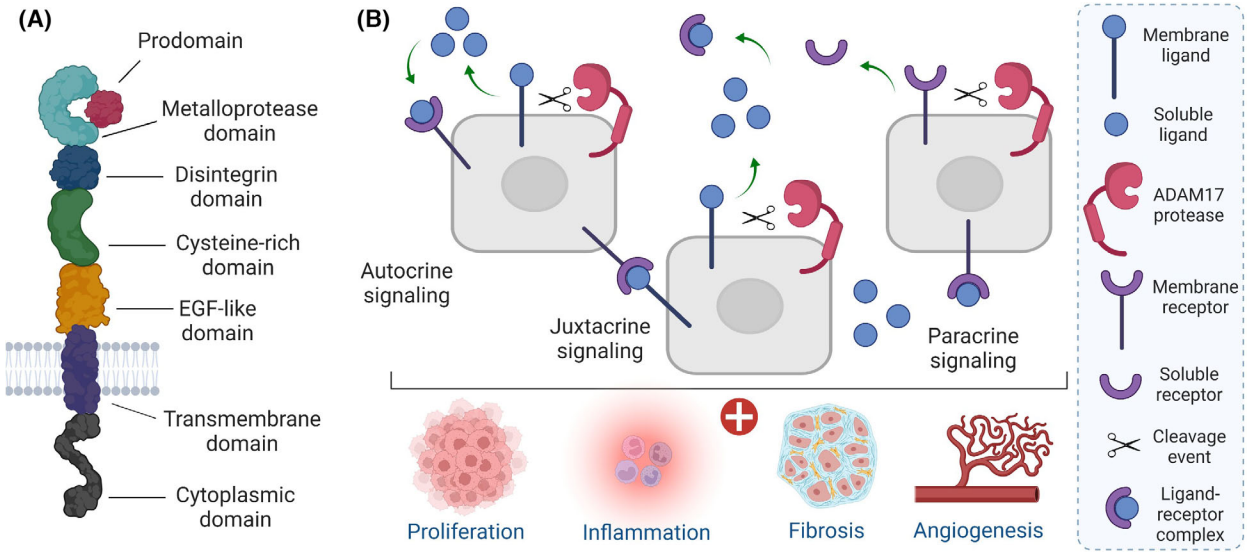

Fig. 1. ADAM17-mediated shedding events. (A) Schematic representation of the structural and functional domains that comprise ADAM17. (B) ADAM17-dependent signaling modes and biological processes. ADAM17 cleaves membrane substrates including ligands and receptors releasing soluble substrates which activate signaling pathways through different signaling modes; autocrine, juxtacrine and paracrine. Soluble receptors can bind to specific ligands to inhibit their biological activity (i.e., decoy receptors) or form a complex with ligands to activate trans-signaling via stimulating co-receptors on distinct cells.

domain of ADAM17 is not required for its rapid response to PMA [31]. The notion that phosphorylation of the ADAM17 cytoplasmic domain may play a pivotal role in controlling its sheddase activity has come from the observation that phosphorylation of the Threonine 735 residue (Thr^{735}) in the cytoplasmic domain of ADAM17 by MAPKs, including extracellular signal-regulated protein kinase (ERK1/2) and p38 MAPK, enhances ADAM17 shedding activity [32–36]. In contrast, the ADAM17 cytoplasmic domain was shown to be dispensable for its post-translational activation by several stimuli including PMA, suggesting that it is not required for ADAM17 induced activity *in vitro* [37,38]. With respect to interacting partners, tissue inhibitor of metalloproteinases-3 (TIMP3) inhibits dimerized ADAM17 activity via association with its ectodomain at the cell membrane [39,40]. Recently, the iRhom tail-associated protein (iTAP) has also been identified as a novel regulator of ADAM17 activity by binding to iRhoms, enhancing the cell surface stability of iRhoms/ADAM17 sheddase complex, and preventing its lysosomal degradation [41,42].

Among ADAM17-deficient mouse models, homozygous *Adam17* gene deletion results in perinatal lethality [18], while the viable $Adam17^{ex/ex}$ mice, which are homozygous for the $Adam17^{ex}$ allele generated via inserting a new exon into the *Adam17* gene resulting in termination of ADAM17 protein translation, develop eye, skin, and heart defects with reduced levels of soluble ADAM17 substrates [9]. Several viable heterozygous and conditional ADAM17 knockout mice with different observed phenotypes have also been generated, which serve as an efficient tool to study the role of ADAM17 in the physiology and pathophysiology of certain mammalian tissues, including the pancreas, various immune cell types, and cardiomyocytes [15–17,21]. These *in vivo* data suggesting the importance of ADAM17 activity for normal development and health have been supported by clinical observations, whereby homozygous and compound heterozygous loss-of-function mutations in ADAM17 in several individuals have been associated with inflammatory skin and bowel lesions, heart conditions, and a predisposition to infections, which collectively can contribute to premature death [43–46].

Through the use of the abovementioned mouse models, ADAM17 has emerged as a key regulator of several physiological and pathophysiological processes, including cell proliferation, immune cell activation, cell death, inflammation, and cancer, owing to its role as a major sheddase of at least 80 known membrane-tethered proteins. These include TNFα, EGFR ligands such as amphiregulin and transforming growth factor α (TGFα), and the interleukin (IL)-6 receptor (IL-6R). With respect to IL-6R, its soluble form (sIL-6R) drives the proinflammatory IL-6 trans-signaling cascade [7,10,18,21,47,48]. Unlike classical IL-6 signaling that controls acute inflammatory and homeostatic

responses induced by binding of IL-6 to the membrane-bound IL-6R on target cells, IL-6 trans-signaling requires the formation of a complex between IL-6 and sIL-6R (shed predominantly by ADAM17), which then binds to the ubiquitously expressed gp130 coreceptor, eliciting expansive cellular responses [49,50]. Both signaling modes initiate the activation of several downstream pathways, including Janus kinase/signal transducer and activator of transcription (JAK/STAT), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), protein kinase B (PKB or AKT), as well as p38 and ERK1/2 MAPKs [49–52].

ADAM17 in inflammatory conditions

Cellular (i.e., macrophages, neutrophils, eosinophils, and lymphocytes) and molecular (i.e., cytokines and chemokines) mediators of inflammation play an integral part in a wide range of mammalian physiological and pathophysiological processes, including tissue damage and remodeling, metabolism, cancer, and infections with microbial pathogens [53]. ADAM17 has been implicated in many inflammatory disorders, mainly through shedding of active cytokines, chemokines, and adhesion molecules, as well as modulating the functions of immune cells (Table 1) [7,54]. From a clinical viewpoint, the expression and/or activation levels of ADAM17 are elevated in patient biopsies of inflammatory conditions, including chronic obstructive pulmonary disease (COPD)/pulmonary emphysema [13], Crohn's disease [55], rheumatoid arthritis [56], pancreatitis [14], and psoriasis [57]. Moreover, proof of concept that targeting ADAM17 can be therapeutically beneficial in immune-based disorders has come from studies employing the genetic and pharmacologic inhibition of ADAM17 in preclinical disease models (Table 1). For example, using the *Adam17*$^{ex/ex}$ hypomorphic mouse strain, which displays significantly reduced mRNA and protein levels of ADAM17, the deficiency of ADAM17 ameliorated lung inflammation and cellular damage in models of COPD/emphysema [13]. Moreover, pharmacologic ADAM17 blockade with nonselective ADAM17/MMP chemical inhibitors (TMI-1, apratastat) prevented leukocyte infiltration and lung injury in a mouse model of coronavirus disease 2019 (COVID-19) [58]. In a human neutrophil elastase-induced mouse model of lung inflammation, conditional deletion of ADAM17 using the Cre-loxP system attenuated goblet cell metaplasia while having minimal effects on tissue inflammation, the latter likely explained by the modest effect of the Cre-loxP system on reducing ADAM17 protein levels in the lung [59]. Together, these observations imply an important role for ADAM17 in promoting dysregulated inflammatory responses in the lung.

A Disintegrin And Metalloproteinase17 also plays a central role in hepatic inflammation, with enhanced ADAM17 activity being a hallmark of hepatic inflammation and injury in animal models and in biopsies of patients with inflammatory liver diseases, such as primary biliary cholangitis [60]. Mice lacking *Timp3*, a specific endogenous inhibitor of ADAM17 activity, serve as a model of ADAM17 gain of function, and exhibit hepatic lymphocyte infiltration and necrosis, mainly due to constitutive ADAM17-dependent TNFα signaling in the liver [60]. Moreover, pharmacologic inhibition of ADAM17 using the selective small molecule inhibitor DPC-333 ameliorated liver injury in a bile duct ligation-induced mouse model of cholestatic liver injury [61].

In the context of intestinal inflammation, the genetic targeting of ADAM17 (*Adam17*$^{ex/ex}$ mice) caused severe weight loss, and exacerbated intestinal inflammatory responses and ulcerations in a dextran sulfate sodium (DSS)-induced colitis mouse model. These ADAM17-dependent anti-inflammatory properties were attributed to failure of TGFα-dependent intestinal epithelial cell regeneration that requires ADAM17-mediated TGFα shedding [9]. By contrast, administration of the specific A17pro inhibitor to mice subjected to 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis model ameliorated the severity of symptoms and improved survival rate of mice [62]. The anti-inflammatory properties assigned to A17pro-mediated ADAM17 blockade in this model were attributed to its preferential accumulation in inflamed colon tissues, and the type of substrate utilized by ADAM17; while cleavage of TGFα is required for homeostatic tissue repair, ADAM17-generated TNFα exacerbates disease pathology [62]. These differences could also be attributed to the intrinsic molecular and cellular characteristics of animal models used. For instance, while DSS-induced colitis is characterized by focal crypt lesions, goblet cell loss and inflammatory cell infiltration at the areas of lesions, TNBS-induced colitis exhibits loss of architecture, and transmural immune cell infiltration in the mucosa and submucosa [63].

The therapeutic effects of ADAM17 blockade have also been demonstrated in polymicrobial sepsis using the caecal ligation and puncture (CLP) mouse model. Here, conditional knockout mice lacking ADAM17 in all leukocytes or treating mice with a novel monoclonal antibody targeting ADAM17 (MEDI3622) improved survival during sepsis, which was associated with augmented neutrophil functions and reduction in

Table 1. Preclinical studies implicating ADAM17 in the pathogenesis of inflammatory disorders.

Disease/ condition	Model(s)	ADAM17 modulation	Substrate (s) involved	Disease outcome(s)	Reference (s)
Emphysema/ COPD	Emphysema-prone *gp130*$^{F/F}$ Acute cigarette smoke-induced lung pathology models	Genetic inhibition of ADAM17 using *Adam17*$^{ex/ex}$ mice	sIL-6R	ADAM17 deficiency: • ameliorated the development of pulmonary emphysema • suppressed elevated alveolar cell apoptosis • reduced pulmonary inflammation	[13]
COVID-19	Mouse model of COVID-19 via intratracheal instillation of poly-I:C and recombinant RBD domain of the SARS-CoV-2 Spike protein	Pharmacologic inhibition of ADAM17 using intraperitoneal injections of non-selective ADAM17 inhibitors, apratastat and TMI-1	None directly measured	Inhibition of ADAM17 protected against lung inflammation and reduced: • mRNA levels of inflammatory genes • neutrophil recruitment to the lung	[58]
Lung neutrophilic inflammation	Human neutrophil elastase-induced mouse model	Conditional deletion of ADAM17	No change in TNFα	ADAM17 deficiency: • ameliorated goblet cell metaplasia • had minimal effects on tissue inflammation	[59]
Cholestasis-associated liver injury	Mouse model of cholestatic liver injury due to bile duct ligation	Pharmacologic inhibition of ADAM17 using intraperitoneal injections of a selective ADAM17 inhibitor, DPC-333	None directly measured	Inhibition of ADAM17 activity improved: • cholestatic liver injury • sickness behavior development	[61]
Pancreatitis	Cerulein-induced acute pancreatitis model Acute and chronic pancreatitis models induced by the cigarette smoke carcinogen nicotine-derived nitrosamine ketone (NNK)	Genetic and therapeutic inhibition of ADAM17 using *Adam17*$^{ex/ex}$ mice and the selective ADAM17 prodomain inhibitor (A17pro), respectively	sIL-6R	Targeting of ADAM17: • ameliorated experimental pancreatitis • reduced inflammatory cell infiltration • reduced pancreatic necrosis and fibrosis	[14]
Sepsis	Murine caecal ligation and puncture model of polymicrobial sepsis	Conditional knockout mice lacking ADAM17 in all leukocytes or targeting ADAM17 using a novel monoclonal antibody, MEDI3622	TNFα	Inhibition of ADAM17 led to: • improved survival during sepsis • reduced levels of proinflammatory cytokines • enhanced neutrophil recruitment	[64,65]
Colitis	Dextran sulfate sodium-induced mouse model 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced mouse model	Genetic deficiency of ADAM17 using *Adam17*$^{ex/ex}$ mice Blocking the activity of ADAM17 using ADAM17 prodomain inhibitor (A17pro)	TGFα TNFα	Genetic inhibition of ADAM17 led to: • severe weight loss, intestinal inflammation and ulcerations • blunted TGFα-mediated intestinal epithelial regeneration Therapeutic inhibition of ADAM17: • improved survival rate • ameliorated the severity of symptoms	[9,62]
Arthritis	K/BxN arthritis mouse model Collagen-induced arthritis (CIA) mouse model	Genetic inhibition of ADAM17 in myeloid cells Blocking the activity of ADAM17 using ADAM17 prodomain inhibitor (A17pro)	TNFα	Inhibition of ADAM17: • ameliorated the severity of inflammatory arthritis	[62,67]

Table 1. (Continued).

Disease/ condition	Model(s)	ADAM17 modulation	Substrate (s) involved	Disease outcome(s)	Reference (s)
Endotoxic shock	LPS-induced mouse model	Genetic ablation of ADAM17 in myeloid cells	TNFα	Inhibition of ADAM17: • protected mice from endotoxic shock	[66]
Diabetes and vascular inflammation	Diabetes-prone *Insr*$^{+/-}$ mice High-fat diet (HFD)-induced model of obesity and insulin resistance	Using *Adam17*$^{+/-}$ mice or the non-selective ADAM17 inhibitor TAPI via intraperitoneal injections	TNFα	Inhibition of ADAM17 led to: • reduced hyperglycemia • diminished vascular inflammation • increased insulin sensitivity	[15,71]
Obesity-induced inflammation	HFD mouse model of insulin resistance	Intraperitoneal injection of non-viral gene delivery system targeting ADAM17 in visceral adipose tissue macrophages	TNFα	Inhibition of ADAM17 led to: • ameliorated systemic inflammation • improved glycaemic control	[74]

proinflammatory cytokine production [64,65]. Similarly, mice lacking ADAM17 in their myeloid cells or treated with A17pro were protected from endotoxic shock induced by injection of lipopolysaccharides (LPS) [62,66]. The anti-inflammatory effects of targeting ADAM17 also extend to arthritis, whereby in K/BxN and collagen-induced arthritis (CIA) arthritis mouse models, ADAM17 deficiency in the myeloid compartment or blockade of ADAM17 activity using A17pro, respectively, protected mice against inflammatory arthritis [62,67].

Obesity-associated complications such as dyslipidemia, insulin resistance, and type 2 diabetes are associated with low-grade inflammation and increased risk of cardiovascular disease due to release of free fatty acids, adhesion molecules, and inflammatory cytokines [68]. In this respect, indirect evidence for a causal role for ADAM17 in diabetes was suggested by the observation that *Timp3*$^{-/-}$ mice showed signs of insulin resistance-induced hepatosteatosis instigated by a high-fat diet (HFD) [69]. Similarly, excessive ADAM17-driven TNFα shedding in *Timp3*$^{-/-}$ mice promoted diabetes, obesity, insulin resistance, hepatic steatosis, as well as vascular and adipose tissue inflammation in the diabetes-prone *Insr*$^{+/-}$ mice and HFD mouse model of insulin resistance, while reducing ADAM17 expression (using *Adam17*$^{+/-}$ mice) or treating *Insr*$^{+/-}$ mice with hydroxamate-based nonselective ADAM17 inhibitor (TNFα protease inhibitor; TAPI) protected HFD-fed mice from diet-induced obesity, insulin resistance, and diabetes complications [15,70,71]. Conversely, transgenic overexpression of *Timp3* in macrophages results in smaller atherosclerotic plaques, improved glycaemic control and insulin resistance, reduced oxidative stress and hepatosteatosis, as well as reduced inflammation, in low-density lipoprotein receptor knockout (*Ldlr*) mouse model of atherosclerosis as well as a HFD mouse model of insulin resistance [72,73]. Interestingly, a nonviral gene delivery system has been developed consisting of an oligopeptide (ATS-9R) that can selectively silence ADAM17 in visceral adipose tissue macrophages, which ameliorated obesity-induced systemic inflammation and improved glycemic control in a HFD mouse model of insulin resistance [74]. Collectively, these findings emphasize the diverse and important *in vivo* roles of ADAM17 in promoting various inflammatory conditions, highlighting its potential as a promising therapeutic target.

ADAM17 in cancer

Over the last decade, a wealth of clinical data has revealed that the shedding activity of ADAM17 is enhanced or its mRNA and/or protein levels are elevated in many human cancers, including nonsmall cell lung cancer (NSCLC; and its major subtype lung adenocarcinoma) [12,75,76], colorectal cancer [77,78], head and neck squamous cell carcinoma (HNSCC) [79], breast cancer [80], hepatocellular carcinoma [81], pancreatic adenocarcinoma [82], and gastric cancer [83]. In lung tissues of NSCLC patients and xenografts, enhanced ADAM17 activity correlated with increased secretion of its substrate sIL-6R in circulation [12], and increased ADAM17 protein expression correlated with poor patient survival [76]. In HNSCC, patients expressing high levels of ADAM17 mRNA showed significantly reduced overall survival compared with those with low mRNA levels [79]. Levels of both active and immature forms of ADAM17 correlated significantly with proliferation index in biopsies of breast

cancer patients [80]. Protein levels of ADAM17 were also identified as independent prognostic factor for patients with gastric cancer [83]. Collectively, these studies suggest that ADAM17 and/or its secreted substrates could be employed as potential disease biomarkers for specific cancer types.

The *in vivo* tumor-promoting role of ADAM17 is also well-supported by many studies examining the effect of modulating ADAM17 expression and activity in preclinical cancer mouse models (Table 2). In colorectal cancer (CRC), ADAM17 deficiency in *Adam17*$^{ex/ex}$ mice reduced intestinal tumor burden in the *Apc*$^{Min/+}$ mouse model by suppressing the EGFR/IL-6 trans-signaling/β-catenin signaling axis [84]. Specifically, EGFR stimulation (by ADAM17-mediated shedding of EGFR ligands such as amphiregulin) of colonic myeloid cells activated the pro-tumorigenic IL-6 trans-signaling pathway via ADAM17-dependent shedding of sIL-6R, leading to tumor progression [84]. Similarly, treating CRC patient-derived xenograft (PDX) models with the ADAM17 inhibitor MEDI3622 suppressed tumor growth *in vivo* [85]. A pivotal role for ADAM17 in driving carcinogenesis in the lung has also been demonstrated. Here, genetic (*Adam17*$^{ex/ex}$ mice) and/or therapeutic (A17pro inhibitor) blockade of ADAM17 suppressed tumor formation in independent NSCLC mouse models, namely the genetically engineered *Kras*^{G12D} mouse strain, cigarette smoke carcinogen (nicotine-derived nitrosamine ketone; NNK)-induced

Table 2. Preclinical studies implicating ADAM17 in the development of tumors *in vivo*.

Disease/condition	Model	ADAM17 modulation	Substrate(s) involved	Disease outcome(s)	Reference(s)
Colorectal cancer	Apc$^{Min/+}$ mouse model	Genetic deficiency of ADAM17 using *Adam17*$^{ex/ex}$ mice	Amphiregulin sIL-6R	Inhibition of ADAM17 led to: • suppressed tumor formation in the Apc$^{Min/+}$ model • inhibition of STAT3 and Wnt pathway components	[84]
Colorectal cancer (CRC)	CRC PDXs model	Intraperitoneal injection of the ADAM17 inhibitor, MEDI3622	None directly measured	Inhibition of ADAM17 led to: • suppressed tumor growth • inhibited growth of liver metastatic lesions	[85]
Lung cancer	*Kras*^{G12D}-induced mouse model Cigarette smoke carcinogen (NNK)-induced mouse model KRAS-mutant PDXs	Genetic deficiency of ADAM17 using *Adam17*$^{ex/ex}$ mice Blocking the activity of ADAM17 using ADAM17 prodomain inhibitor (A17pro)	sIL-6R	Inhibition of ADAM17 led to: • suppressed tumor development • diminished inflammatory responses • no effect on angiogenesis • inhibition of ERK1/2 MAPK pathway	[12,75]
Ovarian cancer	Intraperitoneal xenografts of a human ovarian cancer cell line	Administration of anti-human ADAM17 IgG D1 (A12) antibody via intraperitoneal injection	TNFR1 Amphiregulin TGFα No effect on TNFα	Inhibition of ADAM17 led to: • suppressed tumor development	[86]
Pancreatic cancer	*Pdx1Cre;LSL-Kras*^{G12D};*Trp53*$^{flox/+}$ mouse model	Pancreas-specific *Adam17*$^{\Delta Ex5/\Delta Ex5}$ mice generated via targeting exon 5 of ADAM17 Administration of anti-ADAM17 A9(B8) IgG antibody via intravenous injection	Amphiregulin TNFα	Inhibition of ADAM17 led to: • suppressed tumor development	[16,87]
Tumor metastasis	Murine model of hematogenic metastasis via injecting syngeneic Lewis lung carcinoma or B16F1 melanoma intravenously	Genetic deficiency of ADAM17 using *Adam17*$^{ex/ex}$ mice Blocking the activity of ADAM17 using TAPI-1 or ADAM17 prodomain inhibitor (A17pro)	TNFR1	Inhibition of ADAM17 led to: • suppressed tumor cell metastasis to the lung • reduced TNFR1-dependent tumor cell–induced endothelial cell death	[88]

lung carcinogenesis, and *KRAS*-mutant PDXs [12,75]. In these models, ADAM17 was activated by p38 MAPK-mediated cytoplasmic phosphorylation (Thr^{735}) to selectively shed IL-6R and trigger the IL-6 trans-signaling/ERK1/2 MAPK axis, culminating in deregulated cellular proliferation and tumor formation [12,75]. In ovarian cancer, the anti-human ADAM17 IgG D1(A12) antibody suppressed the growth of intraperitoneal xenografts of a human ovarian cancer cell line *in vivo* via inhibiting ADAM17-mediated shedding of TNFR1, amphiregulin and TGFα, but not TNFα [86]. In a mutant *Kras*-driven pancreatic cancer mouse model, pancreas-specific deletion of ADAM17 or intravenous injection of an anti-ADAM17 A9(B8) IgG antibody inhibited pancreatic tumor formation coincident with suppressed ADAM17-mediated TNFα and amphiregulin shedding [16,87].

Interestingly, the role of ADAM17 in the tumor microenvironment and metastasis has been demonstrated using a murine model of hematogenic metastasis in which syngeneic Lewis lung carcinoma or B16F1 melanoma (i.e., ADAM17-proficient) tumor cells were injected intravenously into immunocompetent wild-type (WT) or $Adam17^{ex/ex}$ mice (i.e., ADAM17-deficient tumor microenvironment). This resulted in the formation of significantly less lung metastases in $Adam17^{ex/ex}$ mice compared with their WT controls, which was attributed to reduced ADAM17-mediated proteolytic shedding of TNF receptor 1 (TNFR1), culminating into diminished TNF-induced endothelial cell death [88]. Notably, treating WT mice with pharmacological inhibitors of ADAM17, TAPI-1 or A17pro, prior to injecting tumor cells inhibited tumor cell metastasis to the lung [88]. Together, these findings highlight the diverse pro-tumorigenic roles of ADAM17 in various cancer types, providing insights into potential therapeutic strategies targeting ADAM17 for cancer treatment.

ADAM17 in cardiovascular diseases

A growing body of evidence suggests that ADAM17 may also play a key role in the pathogenesis of cardiovascular diseases (Table 3). Levels of ADAM17 mRNA were elevated in patients with myocarditis, which correlated positively with left ventricular volume and negatively with left ventricular systolic function in those patients [89], dilated cardiomyopathy, and hypertrophic obstructive cardiomyopathy [90]. Central genetic or pharmacologic blockade of ADAM17 activity in the paraventricular nucleus using ADAM17 siRNA or the ADAM17 inhibitor TAPI, respectively, reduced TNFα-activated signaling pathways to reduce hemodynamic responses, neuroinflammation, sympathetic excitation, left ventricular end-diastolic pressure, pulmonary congestion, as well as cardiac hypertrophy and fibrosis in rats with myocardial infarction-induced heart failure [91,92]. Moreover, cardiomyocyte-specific ADAM17 was shown to be crucial in postmyocardial infarction (MI) recovery, probably via regulating angiogenesis through modulating the TNFα/NF-κB/vascular endothelial growth factor receptor 2 (VEGFR2) pathway in a mouse model of MI remodeling and ischemia–reperfusion injury [17]. Furthermore, ADAM17-mediated angiotensin-converting enzyme type 2 (ACE2) shedding results in decreased membrane-bound ACE2 in the brain, thus promoting the development of neurogenic hypertension in a deoxycorticosterone acetate (DOCA)-salt mouse model of neurogenic hypertension [93]. Specifically, deficiency of ADAM17 in glutamatergic neurons selectively impairs sympathetic outflow and reduces blood pressure in DOCA-salt model of hypertension [94].

Atherosclerosis is a complex inflammatory disease of the arteries driven by genetic and environmental factors and is the main pathological basis of myocardial infarction and stroke. A Disintegrin And Metalloproteinase 17 has been shown to be upregulated in advanced human atherosclerotic lesions and atherosclerotic lesions of atherosclerosis-prone apolipoprotein E-knockout ($ApoE^{-/-}$) mice [95,96]. Interestingly, in the low-density lipoprotein receptor (*Ldlr*)-deficient mouse model of atherosclerosis, ADAM17 can have an atheroprotective role via the shedding of TNFα and TNFR2, thereby preventing overactivation of endogenous TNFR2 signaling in cells of the vasculature [97]. However, a contrasting finding emerged from a study in a rabbit model of atherosclerosis utilizing ADAM17 gene silencing via intraplaque injection of lentiviral-mediated short hairpin RNA (shRNA), which ameliorated abdominal aortic-positive remodeling and attenuated aortic plaque inflammation through inhibition of the ERK1/2 pathway [98]. These contradictory results observed in studies exploring the role of ADAM17 in atherosclerosis could be attributed to several factors, including variations in experimental models, different cellular contexts, and potentially complex interactions within the signaling pathways involved. Despite the complex and multifaceted roles of ADAM17 in cardiovascular pathologies, these findings nonetheless raise the potential of ADAM17 as a therapeutic target in cardiovascular diseases.

ADAM17 substrate selectivity

A Disintegrin And Metalloproteinase 17 exhibits remarkable substrate selectivity through a variety of

Table 3. Preclinical studies implicating ADAM17 in the development of cardiovascular diseases and its complications.

Disease/ condition	Model	ADAM17 modulation	Substrate(s) involved	Disease outcome(s)	Reference (s)
Heart failure (HF)	Myocardial infarction-induced HF via ligation of left coronary artery in rats	Bilateral paraventricular nucleus microinjection of ADAM17 siRNA or intracerebroventricular infusion of TAPI (ADAM17 inhibitor)	TNFα	ADAM17 inhibition reduces: • sympathetic excitation • left ventricular end-diastolic pressure • pulmonary congestion • cardiac hypertrophy and fibrosis	[92]
Post-myocardial infarction (MI) recovery	MI induced by ligation of the left anterior descending artery in mice	Cardiomyocyte-specific ADAM17 genetic knockdown in mice	TNFα	Cardiomyocyte-specific ADAM17 deletion: • suppresses post-MI angiogenesis • does not affect inflammation levels	[17]
Neurogenic hypertension	Deoxycorticosterone acetate (DOCA)-salt mouse model via implanting a DOCA-silicone subcutaneously and replacing drinking water with 1% NaCl solution	ADAM17 siRNA via intracerebroventricular infusion	ACE2	ADAM17 inhibition attenuated: • ACE2 activity in the hypothalamus • DOCA-salt-induced hypertension	[93]
Atherosclerosis	Low-density lipoprotein receptor (*Ldlr*)-deficient mouse model	Hypomorphic *Adam17*$^{ex/ex}$ mice with low residual ADAM17 expression	TNFα TNFR2	ADAM17 deficiency: • Increased atherosclerotic plaque size independent of modulating plasma cholesterol	[97]
Atherosclerosis	Rabbit model of atherosclerosis using balloon-induced abdominal aorta endothelium injury with 1% cholesterol diet for 16 weeks	Intra-plaque injection of lentiviral-mediated shRNA targeting ADAM17	None detected	ADAM17 inhibition at plaque site: • ameliorated abdominal aortic positive remodeling • attenuated aortic plaque inflammation	[98]

mechanisms, including colocalization, differential expression and coregulatory/signaling proteins, across various biological contexts. Colocalization of ADAM17 with specific substrates emerges as a crucial determinant of substrate preference. In the development of lung adenocarcinoma and pancreatitis, ADAM17 demonstrates selective shedding of the pro-tumorigenic and pro-inflammatory receptor IL-6R, partly due to their close proximity within specialized cellular microdomains, culminating in activation of pathological IL-6 trans-signaling [12,14]. Additionally, differential expression patterns of ADAM17 and its substrates play a role in substrate selectivity. For example, in the cardiovascular system, ADAM17 preferentially sheds endothelial adhesion molecules such as E-selectin and vascular cell adhesion molecule 1 (VCAM-1), contributing to vascular inflammation [99]. Activation of coregulatory/signaling proteins also influences ADAM17 substrate specificity. In hepatic inflammation, the association of ADAM17 with TIMP-3 inhibits the cleavage of membrane-bound pro-TNFα, contributing to tissue homeostasis during inflammation [60]. Moreover, the inflammatory defect seen in *Rhbdf2*$^{-/-}$ mice lacking the ADAM17 regulator iRhom2 is mainly attributed to impaired ADAM17-driven release of TNFα from activated immune cells (e.g., macrophages), suggesting a selective role for iRhom2 in the processing of TNFα by ADAM17 during inflammation [100]. Interestingly, the role of iRhom1 and iRhom2 in regulating ADAM17 substrate selectivity has been reported in mouse embryonic fibroblasts (mEFs), whereby mEFs lacking iRhom2 show reduced shedding of several ADAM17 substrates including heparin-binding EGF, epiregulin, and Kit ligand 2, while TGFα shedding is not affected [27]. On the contrary, mEFs lacking iRhom1 showed reduced shedding of TGFα [27]. Recently, it has been proposed

that this iRhom-mediated substrate selectivity of ADAM17 could be explained partly by the differential presentation by iRhom1 and iRhom2 of substrate cleavage sites to active ADAM17 [101]. Notably, ADAM17 substrate selectivity may also be regulated via certain signaling pathways in response to ADAM17 activity inducers, and irrespective of enhanced ADAM17 protease activity. For example, activation of protein kinase Cα (PKCα) and the PKC-regulated protein phosphatase 1 inhibitor 14D enhances ADAM17-mediated cleavage of TGFα, heparin-binding EGF-like growth factor and amphiregulin, while PKC-δ is required for neuregulin release [102]. Moreover, a protein-disulfide isomerase-regulated juxtamembrane segment in the extracellular domain of the ADAM17 has been identified as a sensor that binds IL-6R, but not TNFα, and mediates its shedding [103]. These examples illustrate how different cellular cues collectively influence substrate selectivity, allowing ADAM17 to precisely modulate various signaling pathways in a context-specific manner. Understanding these mechanisms provides valuable insights into the molecular basis of ADAM17-related diseases and paves the way for the development of targeted therapeutic interventions. It also raises the enticing possibility of using disease-specific ADAM17 substrates as biomarkers for disease diagnosis, prognosis, and/or efficacy of ADAM17 therapeutic inhibition.

Future perspectives

Over the last decade, ADAM17 has emerged as a multifunctional and pleiotropic protease that contributes to the pathogenesis of a multitude of disease states. The importance of ADAM17 in tissue and immune homeostasis is also evident, as loss-of-function mutations in ADAM17 render humans and mice susceptible to skin and bowel inflammation, while mice lacking ADAM17 fail to survive due to several developmental defects [9,18,43]. Despite the advances made in our general understanding of the importance of ADAM17 to tissue homeostasis and the pathological consequences that arise following its dysregulated expression and/or activation, the mechanisms governing ADAM17 activation and substrate selectivity, as well as the cellular context and activating stimuli that influence the diverse biological activities of ADAM17, remain ill-defined.

While an impressive amount of preclinical data suggests that targeting ADAM17 presents as an attractive therapeutic approach for numerous disease states, this has yet to translate into its broad clinical implementation against inflammatory diseases and cancer. A contributing factor to the lack of progress of ADAM17 inhibitors in the clinic has been the adverse side-effects and toxicities (e.g., liver) that accompanied the use of early generation small molecular weight chemical inhibitors of ADAM17, which lacked specificity by targeting other proteases (e.g., ADAM10) [104]. However, these earlier studies have since triggered the advent of a new generation of highly specific anti-ADAM17 antibodies and the ADAM17 prodomain inhibitor that show potent efficacy in preclinical disease models, with the promise to further develop and refine for future clinical implementation [12,14,62,65,87]. In considering the targeting of ADAM17 in human disease in the future, a key challenge will be stratifying patients most likely to respond to ADAM17-directed therapies. In this respect, one might consider that soluble substrates released into the circulation by excessive ADAM17 disease-associated protease activity could provide a viable source of surrogate (predictive of activity) biomarkers, as well as indirect therapeutic targets, for ADAM17. One such example is in NSCLC, where ADAM17 was shown to preferentially shed the sIL-6R to drive IL-6 trans-signaling which promoted lung tumorigenesis [12,75]. Here, high serum levels of sIL-6R (detectable by ELISA) correlated with elevated ADAM17 activity, giving rise to the notion that serum (or potentially other liquid biopsies such as sputum) could be used to select for NSCLC patient subsets with high levels of sIL-6R as a biomarker for ADAM17-directed therapy.

Another potential consideration in targeting ADAM17 in the clinic relates to the notion that ADAM17 blockade may also antagonize the activity of potentially many other ADAM17 substrates, which could have detrimental physiological effects, despite observations to the contrary from preclinical mouse models, which tolerate well the administration of the abovementioned ADAM17 antibody-based and prodomain inhibitors.

This invokes the potential of alternative approaches to indirectly target ADAM17 via its pathological processed substrates (such as sIL-6R) or signaling effectors associated with its activation. With respect to sIL-6R and IL-6 trans-signaling, it is also notable that the IL-6 trans-signaling inhibitor olamkicept (sgp130Fc) has shown promising clinical efficacy in inflammatory bowel disease patients, which has mirrored earlier preclinical data on the efficacy of targeting the ADAM17/sIL-6R axis in mouse colitis models [62,105]. It is therefore conceivable that such indirect targeting of ADAM17 via sIL-6R (or for that matter, other substrates) could be efficacious in other ADAM17-associated disease states.

Despite the large array of substrates assigned to ADAM17, it is of note that only a relatively small

number (e.g., IL-6R, EGFR ligands, and TNFα) have been aligned with ADAM17 disease activity (Tables 1–3). Therefore, it remains unclear whether in specific disease settings ADAM17 only employs a limited number of substrates in its pathologic arsenal, or whether other disease-associated ADAM17 substrates are yet to be identified. Regarding the latter, there remains a clear need to better understand the full spectrum of specific substrates harnessed by ADAM17 in a disease and tissue setting, which will ultimately guide the use of safe and efficacious therapies blocking ADAM17 activity. This warrants further research to construct a comprehensive map of the tissue-specific repertoire of ADAM17 substrates and interacting partners (known or novel) *in vivo*, which could be achieved via further investigations utilizing high-throughput techniques such as terminal amine isotopic labeling of substrates (TAILS) N-terminomics proteomics [106].

Acknowledgments

This work was supported by a Lung Cancer Research Program Idea Development Award (LC170062) from the United States Department of Defense awarded to BJJ, as well as the Operational Infrastructure Support Program by the Victorian Government of Australia. BJJ is supported by a National Health and Medical Research Council (NHMRC) of Australia Senior Medical Research Fellowship. Open access publishing facilitated by Monash University, as part of the Wiley - Monash University agreement via the Council of Australian University Librarians.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

MIS and BJJ wrote and edited the manuscript.

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