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STATE-OF-THE-ART REVIEW

A jack of all trades – ADAM8 as a signaling hub in inflammation and cancer

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As a member of the family of A Disintegrin And Metalloproteinases (ADAM) ADAM8 is preferentially expressed in lymphatic organs, immune cells, and tumor cells. The substrate spectrum for ADAM8 proteolytic activity is not exclusive but is related to effectors of inflammation and signaling in the tumor microenvironment. In addition, complexes of ADAM8 with extracellular binding partners such as integrin β-1 cause an extensive intracellular signaling in tumor cells, thereby activating kinase pathways with STAT3, ERK1/2, and Akt signaling, which causes increased cell survival and enhanced motility. The cytoplasmic domain of ADAM8 harbors five SRC homology-3 (SH3) domains that can potentially interact with several proteins involved in actin dynamics and cell motility, including Myosin 1F (MYO1F), which is essential for neutrophil motility. The concept of ADAM8 thus involves immune cell recruitment, in most cases leading to an enhancement of inflammatory (asthma, COPD) and tumor (including pancreatic and breast cancers) pathologies. In this review, we report on available studies that qualify ADAM8 as a therapeutic target in different pathologies. As a signaling hub, ADAM8 controls extracellular, intracellular, and intercellular communication, the latter one mainly mediated by the release of extracellular vesicles with ADAM8 as cargo. Here, we will dissect the contribution of different domains to these distinct ways of communication in several pathologies. We conclude that therapeutic targeting

Abbreviations
ADAM, A Disintegrin and A Metalloproteinase; APP, β-Amyloid precursor protein; BACE, Beta-site APP cleaving enzyme; Bcl-2, B-cell lymphoma 2; CAD, coronary artery disease; CaMBD, calmodulin binding domains; CCL, chemokine (C-C motif) ligand; CD, cluster of differentiation; CHL1, neural cell adhesion molecule L1-like protein; CIP, Cdc42-interacting protein; COPD, chronic obstructive pulmonary disease; CXCL, chemokine (C-X-C motif) ligand; CXCR, CXC chemokine receptors; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EMT, epithelial to mesenchymal transition; ERK, extracellular signal-regulated kinase; ERK, extracellular signal-regulated kinases; EV, extracellular vesicle; FAK, focal adhesion kinase; GEPIA2, gene expression profiling interactive analysis; GRB, growth factor receptor-bound protein; HB-EGF, heparin-binding EGF-like growth factor; hCG, human chorionic gonadotropin; HR, hazard ratios; HTCD, hypertension cardiovascular disease; HUVEC, human umbilical vein endothelial cells; ICAM, intercellular adhesion molecules; IFN, interferon; IL, interleukin; JAK, janus kinase; JNK, c-Jun N-terminal kinase; KL, Kit ligand; Lck, lymphocyte-specific protein tyrosine kinase; LCN, lipocalin; LH, luteinizing hormone; LPS, lipopolysaccharide; LTB, leukotriene; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; Mcl-1, induced myeloid leukemia cell differentiation protein; MMP, matrix metalloproteinase; MPO, myeloperoxidase; MYO1F, myosin 1F; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK cells, natural killer cells; OA, osteoarthritis; PGR, progesterone receptor; PMA, phorbol 12-myristate 13-acetate; PrPC, cellular prion protein; PSGL, P-selectin glycoprotein ligand; SH, Src homology; SNX, sorting nexin; SPP1, osteopontin; TCGA, The Cancer Genome Atlas Program; TGF, transforming growth factor; TIMP, tissue inhibitors of metalloproteases; Tks5, SH3 and PX domain-containing protein 2A; TMZ, temozolomide; TNF, tumor necrosis factor; TOCA, Cdc42-dependent actin assembly protein; VCAM, vascular cell adhesion protein; ZO, zonula occludens.

attempts for ADAM8 should consider blocking more than a single domain and that this requires a thorough evaluation of potent molecules targeting ADAM8 in an *in vivo* setting.

Introduction

A Disintegrin and A Metalloprotease (ADAM) proteins are type 1 transmembrane proteins mainly responsible for the cleavage of different proteins close to the cell membrane in a process called ectodomain shedding [1]. Given their multidomain structure, ADAM proteases execute different functions, either related to their catalytic activity, including substrate recognition, to integrin binding and activation [2,3], or to intracellular signaling via their cytoplasmic domains. Several regulatory checkpoints exist for their expression and subsequent activity. One of the most important ones involves their prodomains, which ensure correct folding of the protease and keep the protease in an inactive state. Only after cleavage of the prodomain at the end of the secretory pathway, usually by furin convertases, the active protease is translocated to the cell surface, where it performs its cleavage functions [4].

In humans, there are 33 known *ADAM* genes, of which 21 are functional. From these, not all possess catalytic activity since only 13 comprise the reprolysin active-site consensus sequence complexing the zinc ion by three histidine residues (HEXGHXXGXXHD) [1]. Two ADAM proteases, ADAM10 and ADAM17, are constitutively expressed in many cell types throughout the body and are essential for homeostasis, as their deficiency causes embryonic (ADAM10) or early postnatal (ADAM17) lethality in mice [5,6]. Further, nearly all membrane proteins known to be shed by ADAM proteases are cleaved by one of these two ADAM proteases. However, several ADAM proteins are expressed under pathological conditions with overlapping substrate spectra to those from ADAM10 and 17. ADAM8, also known as MS2/CD156a, is one such protease, and due to its structural and functional characteristics, ADAM8 can exert multiple (pathological) functions. Since the functions of ADAM8 go far beyond that of a shedding enzyme, we summarize here all observations made in the context of homeostasis and pathology over the last decades.

ADAM8 in homeostasis and inflammation

ADAM8 has some unique properties compared to other members within the ADAM protein family, such as ADAM10 and 17. Among the proteolytically active ADAM family members, only ADAM8 and ADAM28 are known to be activated through autocatalysis, not only during translocation to the cell surface but also during further processing that results in the catalytic domain to be released in a soluble form [7]. However, as a major difference, ADAM8 exerts a distinct expression pattern with low expression levels throughout the organism except for bronchial epithelial cells (three-fold over median expression levels), cells of the lymphoid organs, central nervous system, and of the bone [8–11]. However, ADAM8 was initially identified as MS2/CD156a from macrophages [12,13]. Since then, further studies on ADAM8 in immune cells have been published in which ADAM8 was detected in B cells, $CD4^+$ and $CD8^+$ T-cells, dendritic cells, leukocytes, and natural killer cells (NK cells) [14–18]. While ADAM8 is dispensable for homeostasis, underlined by the lack of a phenotype in unchallenged *Adam8* knockout mice [8], it can be strongly upregulated upon stimulation with inflammatory cytokines, chemokines, and effectors of innate immunity as a result of different inflammatory and neoplastic stimuli (Table 1). Thereby, the expression levels of ADAM8 can reach up to 30-fold over median expression (Fig. 1). Unlike other immune cells, ADAM8 is constitutively expressed in neutrophil granulocytes [17] and abundant in the membranes of all types of granules and on the surface of neutrophils. It can be translocated to and autocatalytically released from the plasma membrane upon neutrophil activation *in vitro* and *in vivo* [19]. ADAM8 expression levels have been reported to be increased in inflammatory foci, and the amount of soluble ADAM8 correlated with the total number of inflammatory cells, especially neutrophils and eosinophils [11,17,20–23]. Interestingly, the migratory ability of leukocytes, monocytes, and T lymphocytes isolated from Adam8 knockout mice was attenuated [19], which was confirmed by intravital microscopy [24]. For most inflammatory diseases, experiments with *Adam8*-deficient mice demonstrated a pathology-promoting effect of ADAM8. For instance, in experimental asthma, ADAM8 led to massive recruitment of immune cells to the airway inflammation site, causing the pathological phenotype [15,25]. In contrast, mice lacking *Adam8* or treated with inhibitors for ADAM8 (BK-1361) [26] or an anti-ADAM8 antibody [25] showed significantly improved pathologies. In another

Table 1. Overview of inflammatory cytokines and chemokines that induce ADAM8 expression under pathological conditions. CNS, central nervous system; EGF, epidermal growth factor; hCG, human chorionic gonadotropin; IFN, interferon; IL, interleukin; LH, luteinizing hormone; LPS, lipopolysaccharide; LTB, leukotriene; PGR, progesterone receptor; PMA, phorbol 12-myristate 13-acetate; TGF, transforming growth factor; TMZ, temozolomide; TNF, tumor necrosis factor.

Inducer	Cells/tissues (disease) affected	References
IL-1β	Hepatocytes	[86]
IL-4	Lung (Asthma)	[11]
IL-13	Lung (Asthma)	[11]
TGF-β	Liver stellate cells	[86]
IFN-γ	Monocytes, macrophages	[13,87]
	Endothelial cells (hepatocellular)	[86]
	Endothelial cells, epithelial cells	[19]
TNF-α	Neurodegenerative disease	[9]
	Eendothelial cells	[86]
	Endothelial cells, epithelial cells	[19]
LPS	Astrocytes, microglia, neurons (CNS inflammation), macrophages, hepatocytes	[9,86,87]
PMA	Translocation of ADAM8 to the cell membrane of neutrophils	[17]
Ovalbumin, diverse allergens	Lung (Asthma)	[11]
LTB B4	Lung (Asthma)	[88]
Fatty acids	Hepatocytes	[86]
LH, hCG, PGR, EGF, Epiregulin	Granulosa cells of preovulatory follicles (murine ovary)	[89]
TMZ	GBM cells and in recurrent GBM tissue	[69]
Hypoxia	Pancreatic cancer cell lines	[90]

pathophysiological context, it was demonstrated that both neutrophils and macrophages are recruited during skeletal muscle regeneration to clear the remnants of destroyed muscle fibers. Data from the skeletal muscle of dystrophin-null mice crossed with *Adam8* knockout mice confirmed that ADAM8 is responsible for infiltrating neutrophils and macrophages into tissue and contributes to successful skeletal muscle regeneration [27]. A list of pathological conditions that benefit from ADAM8-deficiency is shown in Table 2.

Roles of ADAM8 in cancer

Despite a relatively low expression level in most somatic cells, it was intriguing that ADAM8 is significantly upregulated in several tumor pathologies according to The Cancer Genome Atlas Program (TCGA) data from a total of 32 tumor entities (see Fig. 2, Table S1).

In several studies, it was described that tumor cell migration and invasion, key steps in the process of extravasation and metastasis of tumor cells, are dependent on ADAM8 expression [28–30]. Immune cells play an increasingly important role in tumor progression as they are recruited to the tumor site to exert tumor-promoting functions [31,32]. This is further highlighted by the gene expression profiling interactive analysis (GEPIA2) [33] of ADAM8 with various immune cell markers in different tumor entities (Fig. 3, Table S2). Recent immunohistological studies in tumor tissues from pancreatic ductal adenocarcinoma (PDAC) and glioblastoma (GBM) patients revealed ADAM8 expression in tumor-associated macrophages, NK cells, and neutrophils [18,34]. In PDAC, ADAM8-positive neutrophil granulocytes were abundant, particularly in venules crossing the tumor area, and correlated negatively with patient survival. These results suggest that ADAM8 in neutrophils of tumor patient tissues could serve as a potential prognostic factor [18]. The co-culture with M0 macrophages increased the expression levels of lipocalin 2 (LCN2) and the associated activation of matrix metalloproteinase 9 (MMP-9) in PDAC tumor cells via an ADAM8-dependent mechanism. Additionally, both intracellular and soluble ADAM8 levels were upregulated in PDAC tumor cells co-cultured with anti-inflammatory M2-like macrophages, while the ADAM8 dependence of LCN2 expression was abrogated by exposure of pro-inflammatory M1 to PDAC tumor cells. In contrast, the expression of ADAM8 in macrophage-like cells was independent of their polarization status [30], which was confirmed in GBM patients [34]. On a functional level, ADAM8 was also shown to promote angiogenesis in both GBM tumor cells and macrophages by inducing osteopontin (SPP1) through the janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway [35]. In summary, ADAM8 plays a role in tumor-associated immune cells, particularly macrophages and neutrophils, in the context of cancer. Although ADAM8 has been shown to regulate the expression and associated secretion of chemokine (C-C motif) ligand 2 (CCL2), a cytokine known to attract macrophages [36], its exact non-catalytic and catalytic functions in the recruitment of immune cells to the tumor microenvironment (TME) still needs to be conclusively investigated.

Evolution of catalytic vs. non-catalytic functions and binding motifs of ADAM8

The differences in the biological significance of the catalytic and non-catalytic functions were defined for individual ADAM proteins over the last two decades.

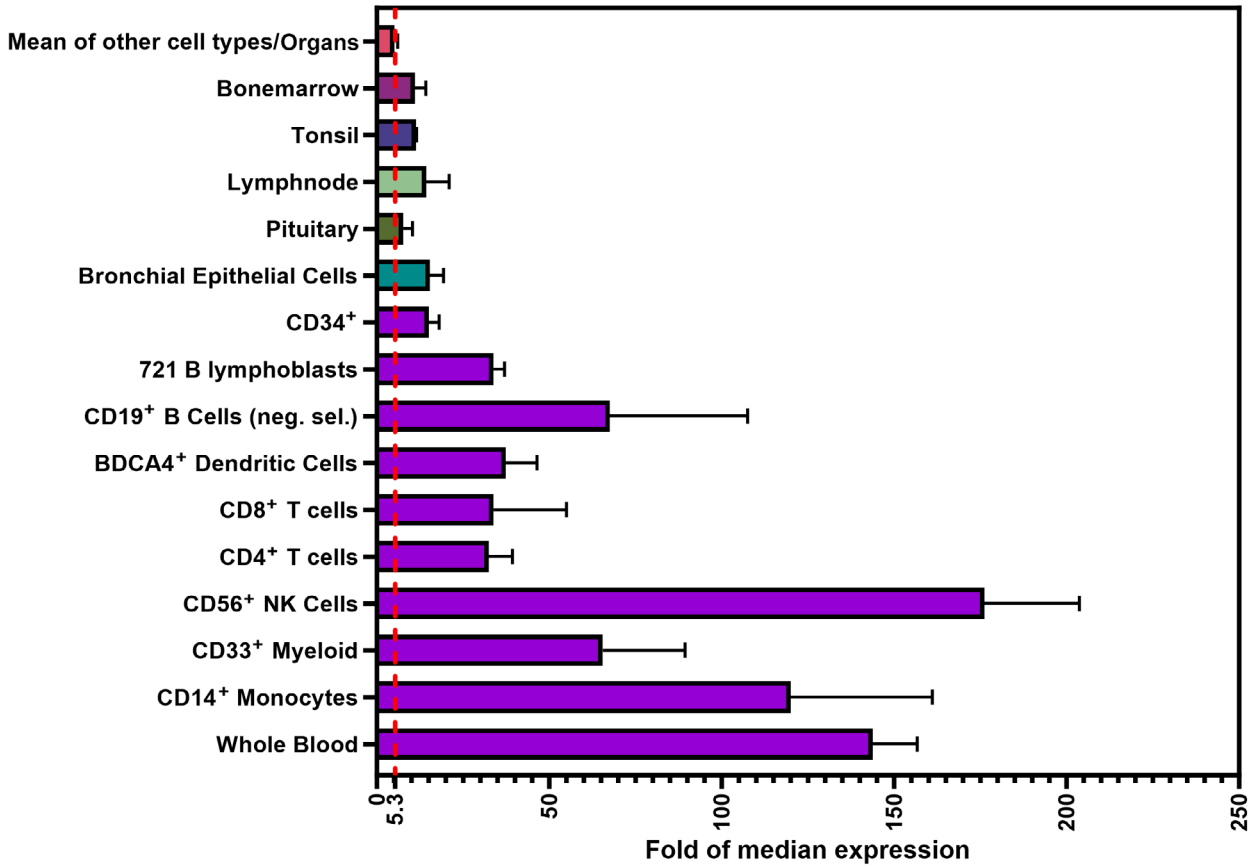

Fig. 1. Organ/tissue distribution of ADAM8 according to BioGPS (http://biogps.org; accessed on October 09, 2023). Note that the 5.3-fold (red dashed line) of the median expression is exceeded only in lymphatic tissue such as lymphnodes, bone marrow, as well as bronchial epithelial cells. However, the highest expression levels of ADAM8 are observed in B cells, myeloid cells, NK cells, whole blood, and monocytes. Data set used: GeneAtlas U133A, gcrma; Probe set used: 205180_s_a [110].

A recent phylogenetic work showed that during evolution, ADAM proteins appeared in the last common ancestor of eukaryotes since many *ADAM* genes were identified in early-branching green algae and greatly expanded during vertebrate development, where gene duplication events led to the addition of more members [37]. This is the first hint to the hypothesis that non-catalytic functions have evolved at the cost of the relevance of proteolytic functions. Here, we focused on phylogenetic trees based on structural (functional) alignment in comparison with mRNA sequences-based trees (see Fig. 4, Tables S3 and S4). Particularly considering that ADAM7 does not possess the zinc-binding consensus sequence and is proteolytically inactive, and although ADAM28, the other cluster member, is, in fact, proteolytically active, the non-proteolytic function of both proteases might still play important roles. For example, an integrin binding study concluded that the disintegrin domains of ADAM7 and 28 are capable of binding to leukocytic integrins α4β1, α4β7, and α9β1 while ADAM33 was only capable of binding to the α9β1 [38]. Another study shows that ADAM7 positively regulates the proliferation and invasion of trophoblast cells in a p38 mitogen-activated protein kinase (MAPK) signaling pathway-related manner [39]. From this, it is reasonable to hypothesize that the proteolytic function of ADAM8 is plainly sequence-related, while the non-proteolytic functions might be more related to the topology of the protein and the local interaction of the different topological domains. To distinguish between those functions related to the proteolytic activity occurring outside of the cell and those involving the C-terminus inside the cell, a cluster analysis was also performed using only the protein sequences from the C-terminal tails of each human ADAM. The obtained phylogenetic tree shows that most ADAMs were not clustered together. Additionally, the use of an *in silico* tool of potential calmodulin-binding domains (CaMBD) demonstrates that only some ADAM members show a

Table 2. ADAM8 dysregulation in inflammatory diseases. CAD, coronary artery disease; CCL, chemokine (C-C motif) ligand; CD, cluster of differentiation; COPD, chronic obstructive pulmonary disease; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinases; IL, interleukin; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; TNF, tumor necrosis factor. ↓: low ADAM8 expression, ↑: high ADAM8 expression.

Organ	Disease	ADAM8	Cell type/tissue	Functional role	References
Lung	COPD	↓	Macrophages, epithelial cells	Promotes COPD	[91]
		↑	Airway epithelial cells, airway smooth muscle cells, infiltrated cells into lung parenchyma, vascular smooth muscle cells, neutrophils	Promotes COPD	[20]
	Asthma	↓	Leukocytes	Promotes clearance of inflammatory cells from the lungs	[88]
		↑	Eosinophils	Migration on periostin in asthmatic airway	[23]
		↑	Eosinophils	Promotes disease	[11]
		↑	Eosinophils	Airway inflammation and bronchoconstriction, CD23 shedding	[92]
		↑	Inflammatory cells	Tissue remodeling	[93]
		↑	Airway epithelial cells, airway smooth muscle cells, macrophages	Not described	[94]
		↑	Airway epithelial cells, airway smooth muscle cells, infiltrated cells into lung parenchyma, vascular smooth muscle cells, eosinophils, neutrophils	Neutrophil migration	[20]
		↑	Macrophages, eosinophils, dendritic cells	Immune cell recruitment	[15]
	Acute lung inflammation	↑	Neutrophils, monocytes, T lymphocytes	Important for the recruitment of neutrophils, monocytes, and T lymphocytes into the inflamed lung tissue, promote the acute phase of lung inflammation	[19]
		↑	Lung dendritic cells, lung parenchyma	Decrease of $CD11c^+$ dendritic cells, lower CCL11 and CCL22 production, decreased eosinophilic inflammation	[25]
		↑	Neutrophils	Endothelial trans-migration of neutrophils	[24]
Joints/ Bone	Rheumatoid arthritis	↑	Neutrophils	L-selectin shedding	[17]
		↑	Macrophages, osteoclasts	Cartilage degradation and bone erosion	[95]
	Osteopenia	↑	Osteoclasts	Blocks the enhanced osteoclast formation in response to TNF-α	[96]
	Osteoarthritis	↑	Chondrocytes	Regulates osteoarthritis progression via EGFR/ERK/NF-κB signaling pathway	[75]
Liver	Nonalcoholic steatohepatitis	↑	Hepatic cells, endothelial cells, stellate cells	ADAM8 induces TNFα and IL-6 mRNA and protein	[86]
	Chronic liver disease	↑	Activated hepatic stellate cells	Extracellular matrix remodeling during fibrosis	[97]
Vasculature	Artherosclerosis	↑	Circulating neutrophils and macrophages	Secretion of inflammatory mediators	[98]
		↑	Endothelial cells and leukocytes	CAD and myocardial function	[99]
Brain	Neurodegeneration	↑	Neurons, astrocytes, microglia	TNF-α mediated cell death	[9]

C-terminal CaMBD while many others do not. Furthermore, when the sequences which contained an optimal score of 9 according to the webserver were utilized to build a phylogenetic tree, a divergent branching was observed, with ADAM8 being clustered together with ADAM15 (see Fig. 5, Table S4). However, information regarding the significance of such motifs for ADAM8 catalytic or non-catalytic functions has not been shown so far. The high disorder of the C-terminal tails of most ADAMs turns a direct functional application

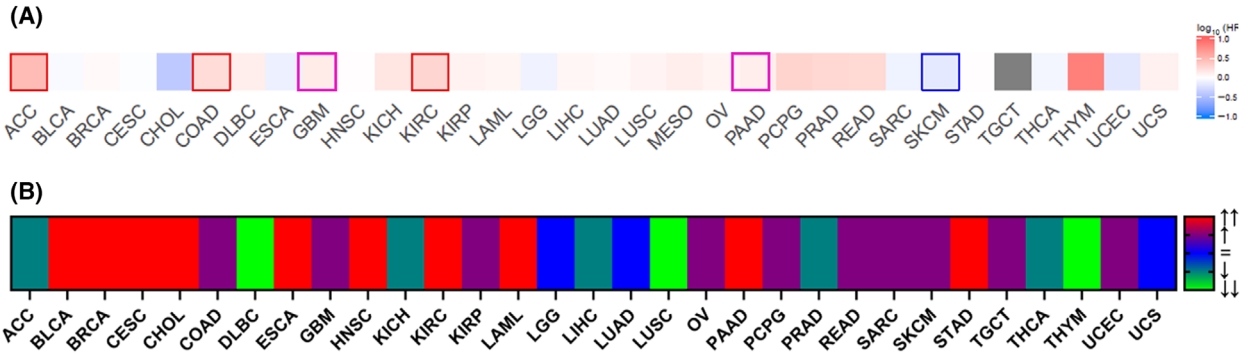

Fig. 2. Survival and expression analysis of ADAM8 in different tumor entities. (A) The displayed survival analysis is based on gene expression performed and provided by gene expression profiling interaction analysis (GEPIA2) http://gepia2.cancer-pku.cn/ (accessed on July 05, 2023). A Mantel-Cox test was used for the data and presented as log 10 of hazard ratios (HR). The significance cut-off level is 0.05. The red color of the boxes indicates a negative correlation, and the blue color has a positive correlation with overall survival. Tumor entities surrounded by red (GEPIA2-based data) and pink (based on Proteinatlas and [64,111]) squares show a significant negative correlation with the overall survival of the respective patients. Tumor entities surrounded by blue (GEPIA2-based data) squares show a significant positive correlation with the overall survival of the respective patients. (B) Heatmap of the tissue-wise expression of ADAM8 in different cancer types. The displayed expression analysis is based on matched TCGA tumor and normal and Genotype-Tissue Expression (GTEx) normal data provided by http://gepia2.cancer-pku.cn/ (accessed on July 04, 2023). *P*-value cut-off: 0.01. The sample numbers used for each evaluation of normal versus tumor samples are listed in Table S1. Red: Significant upregulation of ADAM8 in tumor samples (↑↑). Purple: Tendential upregulation of ADAM8 in tumor samples (↑). Blue: Equal expression of ADAM8 in tumor and normal samples (=). Dark green: Tendential downregulation of ADAM8 in tumor samples (↓). Light green: Significant downregulation of ADAM8 in tumor samples (↓↓). ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic ductal adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma.

rather speculative. Thus, these aspects have to be assessed in future studies.

Altogether, the different cluster analyses showed a higher divergence in the C-terminal domains, supporting the hypothesis of a divergent evolution of non-catalytic functions and the importance of the intracellular binding motifs and their arrangement properties of ADAM8 for signaling processes.

Extracellular signaling

Extracellular signaling of ADAM8 may occur through proteolytic cleavage, cell-matrix interactions, and interactions with integrins. In contrast to the well-studied ADAMs 10 and 17, ADAM8 has no unique cleavage substrate but rather an overlap with other ADAMs or matrix metalloproteinases for which, in most cases, certain conditions are complied (Table 3). Due to the induced expression primarily in cells of the immune system, research on the proteolytic activity of ADAM8 has been focusing on inflammatory responses. Here, one essential substrate is CD23, which is only cleaved by ADAM8 and ADAM15 [40] under inflammatory conditions, while constitutive shedding by ADAM10 was observed [41]. The amount of soluble ADAM8, which is catalytically active, has been shown to be correlated with the amount of soluble CD23 in patients with eosinophilic pneumonia [22]. ADAM8-dependent shedding of vascular cell adhesion protein 1 (VCAM-1) and its interaction with integrin α4β1 could regulate the inflammatory response in allergic lung disease [21]. Besides, ADAM8 can cleave tumor necrosis factor α (TNFα), which in turn enhances the expression of ADAM8 as a positive feedback loop further increased by other TNFα-cleaving proteases [9,40]. On the other hand, ADAM8 activity is linked to a negative regulation of the inflammatory response based on the release of soluble interleukin 1 receptor (IL-1R), a decoy for other cytokines of the same family [42]. Also, adhesion molecules such as L-selectin as well as P-selectin glycoprotein ligand 1 (PSGL-1) are reported to be regulated by ADAM8-mediated shedding [17,43]. Tissue inhibitors of metalloproteases (TIMPs) have been shown to act on ADAMs such as ADAM10 and ADAM17 [44].

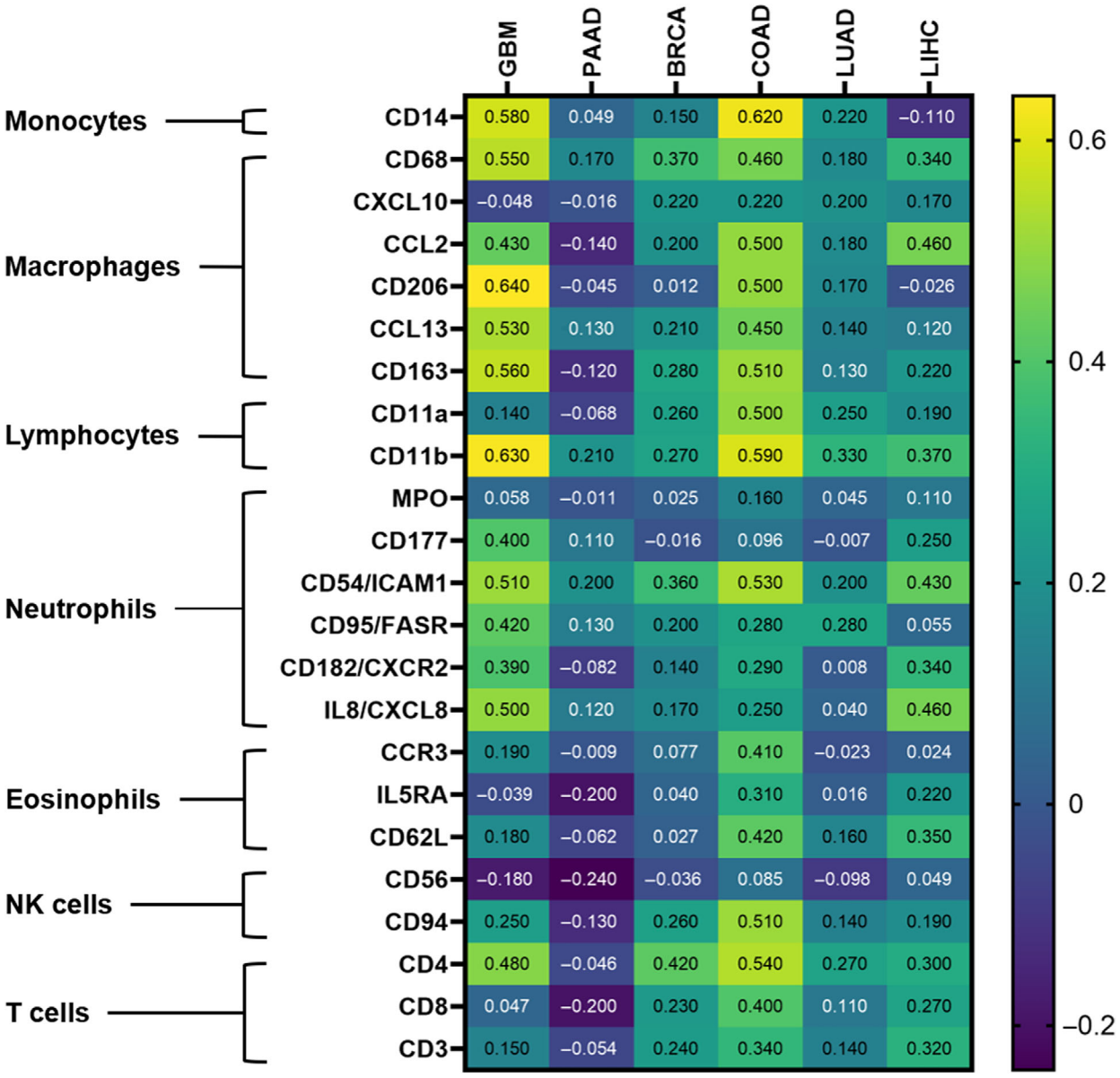

Fig. 3. Heatmap showing the results of a Pearson correlation analysis of ADAM8 with different immune cell markers in various tumors. The displayed correlation analysis is based on TCGA data provided on http://gepia2.cancer-pku.cn/ (accessed on October 10, 2023). The values presented are the generated *R*-values by GEPIA2. Immune cell types are indicated on the left, with square brackets used to categorize each marker. The corresponding p-values are shown in Table S2. BRCA, breast invasive carcinoma; CCL, chemokine (C-C motif) ligand; CD, cluster of differentiation; COAD, colon adenocarcinoma; CXCL, Chemokine (C-X-C motif) ligand; CXCR, CXC chemokine receptor; FASR, Fas receptor; GBM, glioblastoma multiforme; ICAM, intercellular adhesion molecule; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; MPO, myeloperoxidase; PAAD, pancreatic adenocarcinoma.

However, no activity against ADAM8 was observed. Thus, the strong up-regulation in inflammatory settings may lack an endogenous control, highlighting the relevance of ADAM8 for disease progression [44]. Furthermore, *in vivo* experiments using chimeric mice, for instance, by reimplanting Adam8^{+} bone marrow in Adam8-deficient mice after asthma induction, suggest that ADAM8 can exert the observed effects on inflammation and cancer by systemic release. This release from cell types in the microenvironment leads to activity in *trans*, which means on other cells. The systemic function of ADAM8 can be explained by the release of ADAM8 itself from the cell surface, either as part of the secretome [28] or sorted by ADAM8-expressing

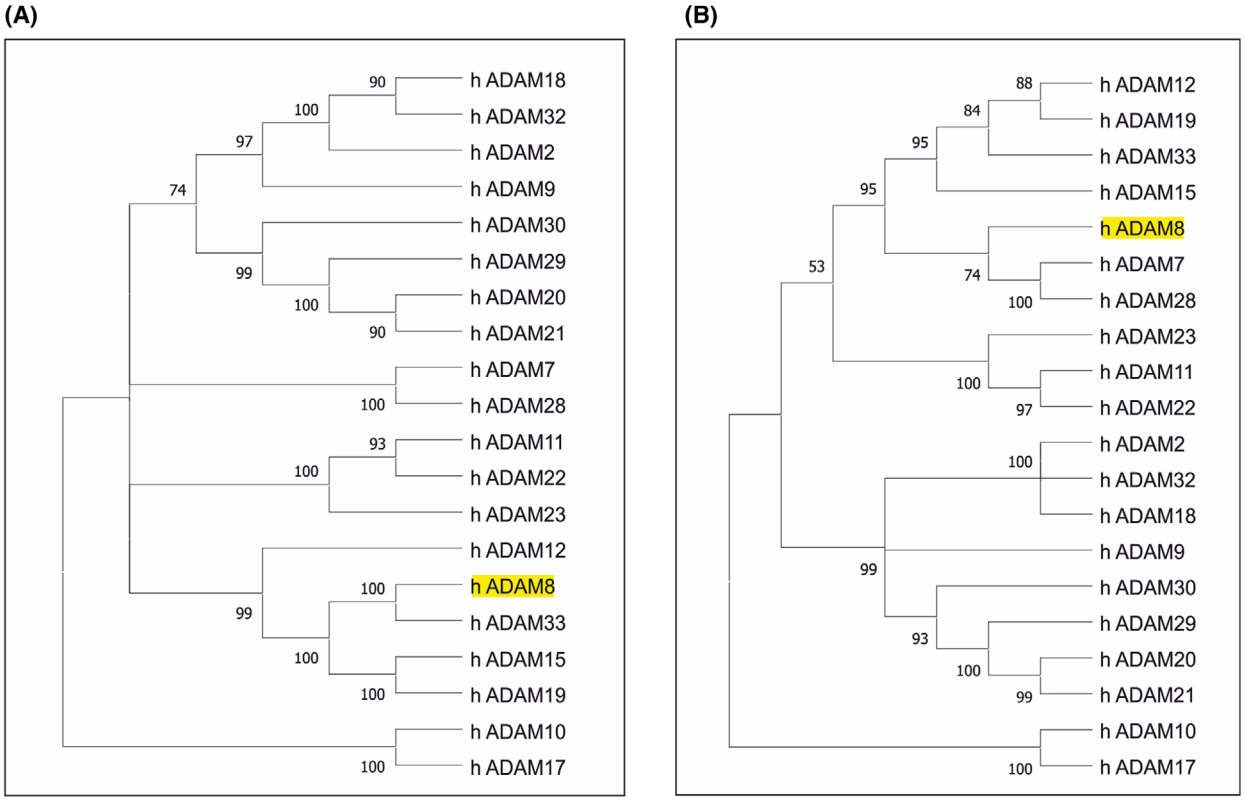

Fig. 4. Multiple alignment and phylogenetic analysis of the ADAM family members. Multiple alignment in (A) was performed based on the mRNA sequences of human ADAM family members using MEGA11 software, while in (B), this was done using the Expresso function of the T-Coffee Multiple Sequence Alignment Server, which can combine protein sequence information with protein structural information [112] and then exported to MEGA11 software. The corresponding accession numbers for the used sequences are shown in Tables S3 and S4. The evolutionary history was inferred using the Neighbor-Joining method [113]. (A) Phylogenetic tree based on a simple mRNA sequence analysis. (B) Structure alignment analysis. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches in both sub-figs [114]. The evolutionary distances were computed using the Jukes-Cantor method [115] (and are in the units of the number of base substitutions per site). This analysis involved 20 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option), and the cluster cut-off value was set to ≥ 50 for the building of a condensed bootstrap tree. There were a total of 11 347 positions in the final dataset for (A) and 1458 for (B). Evolutionary analyses were conducted in MEGA11 [116].

cells in extracellular vesicles (EVs), small double-layered 60–100 nm diameter vesicles with a defined protein corona and cargo. ADAM8 is a cargo of EVs released from tumor cells which are of unknown functional relevance but an excellent analyte for the detection of cancers with high ADAM8 expression levels. Thus, it is required to illuminate the importance of the extracellular interactions and the signaling cascades induced by ADAM8 in more detail.

Cell–cell/cell-matrix and integrin interactions

The disintegrin- and cysteine-rich domains are responsible for several non-catalytic actions in the ADAM8 signaling hub, for instance, they are required for the multimerization step during the autocatalytic processing of ADAM8 [45]. A potential role of the disintegrin-and cysteine-rich domains in cell–cell and cell-matrix interactions has been already described decades ago [46,47]. Cell-adhesive properties include the interaction of these domains with syndecans and ephrins [48]. It was observed that ADAM8 is involved in the fusion process of osteoclastogenesis through binding to integrins such as α9β1 and the formation of docking protein complexes [10,49,50]. Several studies reported a general interaction of ADAM8 with integrin β-1 regulating (a) the adhesion of breast cancer cells (MDA-MB-231) to the endothelial cell layer and subsequent intravasation [51] and (b) polarization and migration of pancreatic ductal adenocarcinoma cells. The binding to integrin subunit β-1 causes integrin activation in an RGD-independent manner (Cook

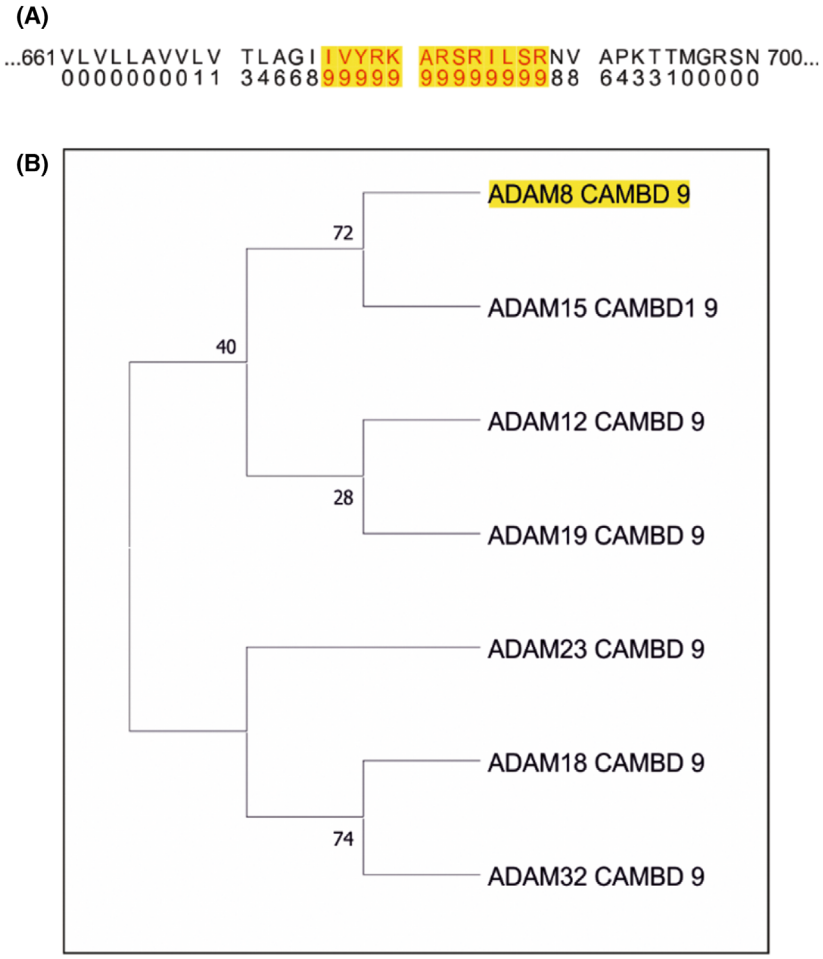

Fig. 5. Calmodulin binding site prediction. (A) ADAM8 sequence was introduced in "The Calmodulin Target Database" [117]. High scores of C-terminal fragment residues close to the transmembrane domain and its scores are highlighted in red. (B) ADAM protein sequences were introduced to the "The Calmodulin Target Database" and only sequences with a score of 9 were introduced to generate the tree. The corresponding accession numbers for the used sequences are shown in Table S4. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [114]. A p-distance substitution model was applied for the analysis. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 40 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [116].

and Bartsch, unpublished data; [29]). ADAM8-integrin interactions were not only reported for cancer cells but also for immune cells. ADAM8 is required for the chemokine-induced upregulation of integrin subunit αL, potentially increasing endothelial cell adhesion via αL–β2 chain ICAM binding [19]. With its proteolytic activity, ADAM8 can not only cleave membrane proteins involved in inflammation and in cancer progression (Table 3), but also extracellular matrix proteins such as fibronectin. As a consequence, fiber displacements in the course of cell migration are dependent on the presence of ADAM8 [52].

Besides the direct interaction with extracellular matrix molecules, non-catalytic functions may also be involved in matrix-modifying processes. Thus, potential treatment options could include the disturbance of the disintegrin/cysteine-rich domain interactions. Indeed, in several studies regarding acute and chronic inflammatory diseases as well as cancer formation, either the use of an antibody directed against the disintegrin domain or the peptide-inhibitor BK-1361 was able to block cell adhesion, integrin relocalization, and cell migration with beneficial disease outcome [7,19,24,26,29].

Intracellular signaling of ADAM8

ADAM8 interacts with other proteins via the cytoplasmic domain

The biological functions of the intracellular cytoplasmic domains have often been neglected and seem to play important regulatory functions in some ADAMs through the binding of calmodulin [53] or SH3-dependent phosphorylation [54]. One important axis for outside-in signaling events is the interaction of ADAM8 with integrins. In pancreatic ductal adenocarcinoma, the binding of ADAM8 to integrin β-1 via its extracellular disintegrin domain has been shown to activate ERK1/2, which could be blocked by an inhibitory peptide mimicking the integrin-binding loop of ADAM8. The resulting intracellular signaling depends

Table 3. ADAM8 substrates. ADAM, a disintegrin and metalloproteinase; APP, β-amyloid precursor protein; BACE, beta-secretase; CD, cluster of differentiation; CHL1, neural cell adhesion molecule L1 like protein; IL, interleukin; KL, kit ligand; MBP, myelin basic protein; MMP, matrix metalloproteinase; PMA, phorbol 12-myristate 13-acetate; PrPC, cellular prion protein; PSGL, P-selectin glycoprotein ligand; TNF, tumor necrosis factor; VCAM, vascular cell adhesion protein.

Substrate	State	Shared metalloprotease	Models and systems used to determine ADAM8 relevance	References
CD23	Induced (ADAM8-dependent), IL-4 induced	ADAM15, ADAM10	*In situ* cleavage assay; purified recombinant ADAM8 from Sf9 cells supernatant	[40,41]
IL-1R	Induced (ADAM8-dependent)	ADAM17	*In situ* cleavage assay; purified recombinant murine ADAM8 from mouse myeloma cell line or *E. coli*. transformation	[44,100]
KL	Induced (ADAM8-dependent)	ADAM9, ADAM17, ADAM33 MMP14, MMP17	*In situ* cleavage assay; purified recombinant murine ADAM8 from mouse myeloma cells or *E. coli* clones	[44,100]
TNFα	Induced (ADAM8-dependent)	ADAM9, ADAM10, ADAM17, ADAM19 MMP17	*In situ* cleavage assay; purified recombinant murine ADAM8 from mouse myeloma cells or *E. coli* clones	[44,100]
APP	Induced (ADAM8-dependent)	ADAM9, ADAM10, MMP14, MMP17	*In situ* cleavage assay; purified recombinant murine ADAM8 from mouse myeloma cells or *E. coli* clones	[44,100]
MBP	Induced (ADAM8-dependent)	ADAM10, ADAM28	*In situ* cleavage assay; purified recombinant murine ADAM8 from mouse myeloma cells or *E. coli* clones	[44,100]
CHL1	Induced (pervanadate, PMA)	(BACE)	*In situ* cleavage assay with lack of batimastat inhibition; purified recombinant from COS-9 cells Adam8 deficient mice; lack of CHL1 processing	[101]
VCAM-1	Induced (TNF, asthma mouse model)	ADAM9, ADAM17	Cell-based cleavage assay; HUVECs incubated with serum obtained from non-transgenic and transgenic mice model	[21,102,103]
L-Selectin	Induced (neutrophil activation – inflammatory response)	ADAM17	Cell-based cleavage assay; comparison of HEK239 cells expressing wildtype ADAM8 or an inactive mutant	[5,17,100]
PSGL1	Induced (activated endothelium)	BACE1, ADAM10	Cell-based cleavage assays; cleavage in neutrophils upon proteolytic activation of ADAM8; comparison of ADAM8, inactive mutant and ADAM8 siRNA knockdown in J77 and HL-60 cells	[28,43,104,105]
Fibronectin	Induced (osteoarthritis chondrocyte conditioned medium)	MMP1, MMP3, MMP13, MMP14	*In situ* cleavage assay; recombinant ADAM8 *ex vivo* protein correlation; human articular cartilage	[106]
PrPC	Induced (Thermolysin ADAM8 activation)	ADAM9, ADAM10, ADAM17	*Adam8*-knockout mice; decrease of C1 fragment and full length ratio of PrPC *in vitro* protein expression; linear correlation of ratio and ADAM8 in proliferating mouse C2C12 myoblasts	[107]

on the presence of the cytoplasmic domain of ADAM8, highlighting the importance of its sheddase-independent structures [29]. In a previous study, five SH3 domains were found at the C-terminal portion of ADAM8. A conducted screen based on phage display determined strongly selected binding partners for ADAM8, such as sorting nexin 33 (SNX33), Cdc42-dependent actin assembly protein 1 (TOCA1), Cdc42-interacting protein 4 (CIP4), SNX9, Tec protein tyrosine kinase (Tec), and tyrosine kinase Src (Src) [54]. As part of the sorting nexin superfamily, it has been previously described that SNX9 is involved in membrane remodeling, actin assembly, endocytic, and protein recruitment processes, whereas SNX33 appears to be involved in the α-secretase cleavage process of the amyloid precursor protein and the dynamics of actin polymerization [55–60]. In the case of ADAM8, the binding of sorting nexin proteins suggests that the occurrence of ADAM8 in the cell plasma membrane may be partly due to translocation via SNX9 and SNX33. Although TOCA1, which contains an F-BAR domain, is also involved in endocytic trafficking processes, it has been associated with affecting actin dynamics in invadopodia protrusions in breast cancer

cells [61]. Of particular interest, ADAM8 shows a high binding affinity for Src, which is also activated via ADAM8 expression and the interaction with integrin β-1/focal adhesion kinase (FAK) in hepatoma cells [62,63]. Nevertheless, further studies are needed to clarify the functional role of the interaction of ADAM8 with the described proteins above.

As previously described, high ADAM8 expression in neutrophil granulocytes has been shown to promote disease progression. Previous studies have focused on the protease activity of ADAM8, as Gómez-Gaviro demonstrated an ADAM8-dependent regulation of L-selectin shedding to control endothelial rolling and adhesion of leukocytes [17]. However, in a recent study, a novel interaction of the cytoplasmic domain of ADAM8 with the SH3 domain of a cytoskeletal motor protein called Myosin 1F (MYO1F) could be identified in neutrophils. This interaction appears to be indispensable for the transmigration and interstitial migration of neutrophils *in vitro* and *in vivo*. Whereas classical dogma assumes that neutrophils transmigrate through the endothelial barrier by detaching adhesion molecules, proteolysis-independent extravasation via tethering to the cytoskeleton was described [24].

ADAM8 is involved in protein regulation

Dysregulated ADAM8 expression is associated with inflammatory diseases and cancer by regulating proteins involved in the proliferation, migration, and invasion of different types of cells (Table 4). In GBM, the most lethal brain tumor, ADAM8 is highly upregulated and correlates with a poor patient prognosis [64]. A correlation analysis conducted with GEPIA2 revealed a moderate correlation of ADAM8 with STAT3 in GBM (Fig. 6, Table S5). Interestingly, ADAM8 was reported to promote proliferation and invasion by activating STAT3 in GBM cells and macrophages [35,65]. The phosphorylation of STAT3 in GBM cells increased the expression of MMP-9, a metalloprotease known to enhance proliferation and invasion not only in gliomas [65–67]. In addition, ADAM8-dependent phosphorylated STAT3 levels were also found to be responsible for the increased expression of SPP1 and positively correlated with an upregulated tube formation of human umbilical vein endothelial cells (HUVEC). These findings revealed a novel mechanism by which ADAM8 promotes tumor progression by upregulating angiogenesis in tumor and

Table 4. Protein signaling regulated by ADAM8. Akt, protein kinase B; Bcl, B-cell lymphoma; CC, colon cancer; CCL, chemokine (C-C motif) ligand; CSC, chondrosarcoma; EGFR, epidermal growth factor receptor; EMT, epithelial to mesenchymal transition; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GBM, glioblastoma multiforme; GC, gastric cancer; HB-EGF, heparin-binding EGF-like growth factor; HC, hepatoma carcinoma; HTCD, hypertension cardiovascular disease; IL, interleukin; Mcl, induced myeloid leukemia cell differentiation protein; MMP, matrix metalloproteinase; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NSCLC, non-small-cell lung cancer; OA, osteoarthritis; PDAC, pancreatic ductal adenocarcinoma; SPP1, osteopontin; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TNBC, triple-negative breast cancer; TNF, tumor necrosis factor. ↑: Upregulation, ↓: Downregulation.

Signaling protein	Activation	Disease	Downstream	Functional relevance	References
STAT3	pSTAT3 (Y705)	GBM	MMP-9 ↑, SPP1 ↑	Proliferation ↑, angiogenesis ↑	[35,65]
		NSCLC	Bcl-2 ↑, Mcl-1 ↑	Chemoresistance ↑	[68]
ERK1/2	pERK1/2 (T202/Y204)	PDAC	Activity of MMP-2, 14 ↑	Invasion ↑, metastasis ↑	[29]
		GBM	Not described	Chemoresistance ↑	[69]
		TNBC	miR-720 ↑, MMP-9 ↑	Invasion ↑, migration ↑, proliferation ↑	[70]
		GBM	MMP-9 ↑, miR-181a-5p ↓	Proliferation ↑	[65]
		GC	Not described	Invasion ↑, migration ↑, proliferation ↑	[108]
		OA	MMP-9 ↑, TNF-α ↑, IL-6 ↑ Collagen II ↓, Aggrecan ↓	Proliferation ↓, apoptosis ↑	[75]
EGFR	HB-EGF Ligand	GBM	ERK1/2 ↑, Akt ↑, CCL2 ↑	Chemoresistance ↑, Recruitment of macrophages	[36]
FAK	pFAK(Y397)	PDAC	Activity of MMP-2, 14 ↑	Invasion ↑, metastasis ↑	[29]
		HC	Small GTPase ↑	Migration ↑, proliferation ↑, apoptosis ↓	[62]
Akt	pAkt (S473)	GBM	MMPs ↑	Chemoresistance ↑	[69]
		PDAC	Activity of MMP-2, 14 ↑	Invasion ↑, metastasis ↑	[29]
Src	pSrc (Y416)	HC	Small GTPase ↑	Migration ↑, proliferation ↑, apoptosis ↓	[62]
Smad2/Smad3	TGF-β1 Ligand	HTCD	EMT marker ↑	Cardiac fibrosis ↑	[73]
		CC	EMT marker ↑	Invasion ↑	[72]
NF-κB	pNF-κB (S536)	CSC	MMP13 ↑	Invasion ↑, migration ↑	[109]
		OA	MMP-9 ↑, TNF-α ↑, IL-6 ↑ Collagen II ↓, Aggrecan ↓	Proliferation ↓, Apoptosis ↑	[75]

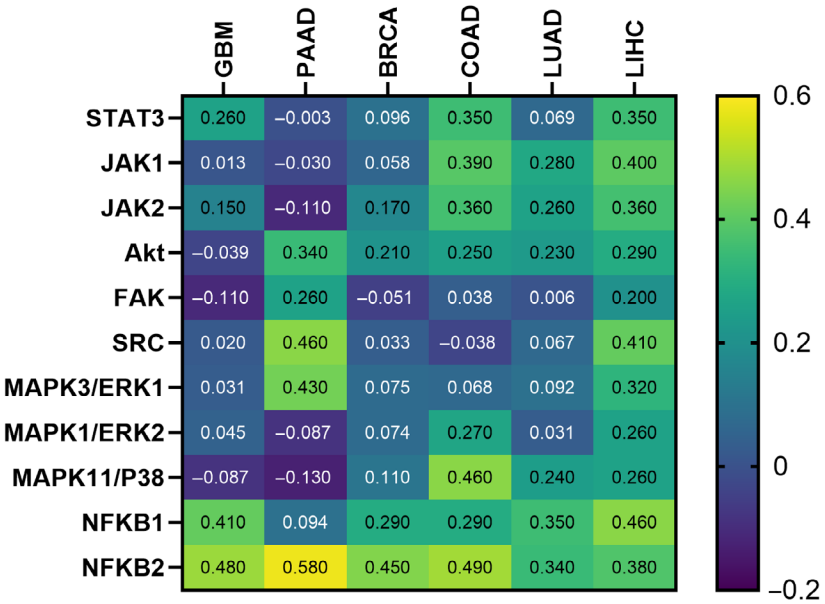

Fig. 6. Heatmap of a conducted Pearson correlation analysis of ADAM8 with different published proteins in various cancer diseases. The displayed correlation analysis is based on TCGA data provided by http://gepia2.cancer-pku.cn/ (accessed on October 10, 2023). The values are the generated R-values by GEPIA2. The corresponding p-values are shown in Table S5. Akt, protein kinase B; BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GBM, glioblastoma multiforme; JAK, Janus kinase; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; MAPK, mitogen-activated protein kinase; NFKB, Nuclear factor kappa-light-chain-enhancer of activated B cells; PAAD, pancreatic adenocarcinoma.

tumor-associated cells like macrophages. Importantly, using specific domain variants of ADAM8, a dependence on its cytoplasmic domain in this type of regulation could be shown [35,65]. The association between ADAM8 and STAT3 activation has also been described in the context of chemoresistance in lung non-small-cell lung cancer. The overexpression of ADAM8 increased the phosphorylation of STAT3 and the resistance of lung cancer cells to cisplatin. Similarly, subsequent silencing of STAT3 reduced the protein levels of the anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2) and induced myeloid leukemia cell differentiation protein 1 (Mcl-1), as well as the susceptibility to chemotherapy [68]. ADAM8 binds to integrin β-1 to activate the intracellular cascade via its cytoplasmic domain in pancreatic cancer. As a result, increased protease activity of MMP-2 and MMP-14 was detectable, and the invasiveness and metastasis of PDAC cells increased *in vitro* and *in vivo* [29]. Following these lines, inhibition of ADAM8 reduced phosphorylated ERK1/2 levels in PDAC, which was later shown to enhance chemoresistance and proliferation in GBM [69]. Further, in both GBM and triple-negative breast cancer, ADAM8-dependent phosphorylation of ERK1/2 led to the induction of MMP-9 expression and concomitant increase in proliferation, migration, and invasion of the respective tumor cells, which was regulated by its cytoplasmic domain [28,65,70].

Phosphorylation of FAK in PDAC was shown to be dependent on the interaction of ADAM8 with integrin β-1and the C-terminal tail of ADAM8 in the same manner [29]. This was confirmed in liver cancer, in which phosphorylation of Src and the activation of small GTPase Rho as downstream signaling cascades involved in migration and cytoskeletal dynamics were positively correlated with ADAM8 expression ([98]; for correlation analysis, see Fig. 6, Table S5).

A further study on GBM assumed that ADAM8 activates epidermal growth factor receptor (EGFR) signaling by regulating the level of heparin-binding EGF-like growth factor (HB-EGF) expression rather than releasing it through shedding events [36,71]. In colon cancer as well as in hypertensive cardiovascular disease, ADAM8 was shown to induce epithelial-to-mesenchymal transition (EMT) via upregulation of the Smad2/Smad3 signaling pathway [72,73]. In the case of colon cancer, overexpression of ADAM8 downregulated E-cadherin and simultaneously upregulated vimentin, N-cadherin, and invasion of colon cancer cells (for confirming correlation analysis including further EMT markers and tumor entities, see Fig. 7, Table S6). In hypertensive cardiovascular disease, upon angiotensin II stimulation, ADAM8 was upregulated and induced EMT ADAM8-dependently via the transforming growth factor β1 (TGF-β1)/Smad2/Smad3 signaling pathway *in vitro* and *in vivo* [73]. Upstream proteins of TGF-β1 are Ras and MAPK, p38 MAPK, ERK and c-Jun N-terminal kinase (JNK), and the PI3K/Akt and RhoA [74], which were all shown to be related to non-catalytic functions of ADAM8, thus once more highlighting the relevance of these intracellular signaling events.

In chondrosarcoma, ADAM8 was reported to increase cell migration and invasion via activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and MMP-13, while silencing of

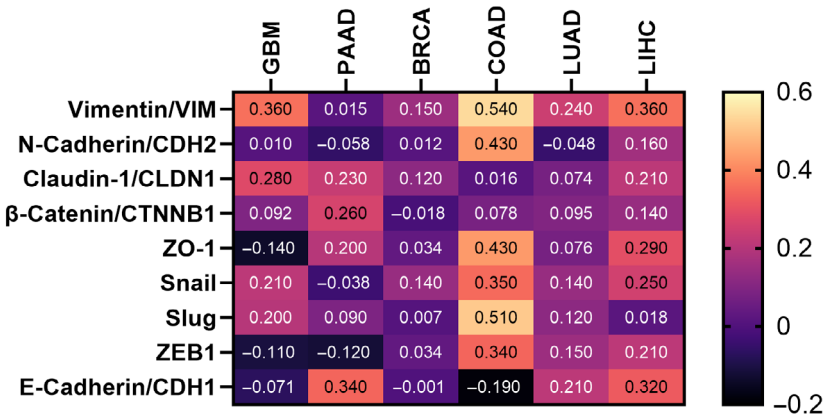

Fig. 7. Heatmap of a conducted Pearson correlation analysis of ADAM8 with different EMT markers in various cancers. Correlation analysis is based on TCGA data provided by http://gepia2.cancer-pku.cn/ (accessed on October 10, 2023). The values are the generated R-values by GEPIA2. The corresponding p-values are shown in Table S6. BRCA, breast invasive carcinoma; CDH, Cadherin; CLDN, Claudin; COAD, colon adenocarcinoma; CTNNB, β-Catenin; GBM, glioblastoma multiforme; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; PAAD, pancreatic adenocarcinoma; VIM, Vimentin; ZEB1, Zinc finger E-box-binding homeobox; ZO, zonula occludens.

ADAM8 in an osteoarthritis cell model led to a decreased ERK1/2 and NF-κB activation, followed by lower levels of MMP-9, TNF-α, and IL-6 expression, increased collagen II and aggrecan expression, higher proliferation rates, and inhibition of apoptosis [75]. LCN2, a downstream protein of NF-κB, positively correlates with ADAM8 expression in PDAC cells and tumor-associated macrophages. LCN2 is a protein that increases MMP-9 activity [30,76,77]. In addition to these findings, ADAM8 expression highly correlates with NFKB2 in PDAC (see Fig. 6, Table S5). Thus, the regulation of protein expression by ADAM8 adds one further trait to the ADAM8 signaling hub.

ADAM8 regulates miRNA expression

MicroRNAs (miRNAs) are small non-coding RNAs consisting of 18–22 nucleotides involved in transcriptional regulation via binding to the 3′-untranslated region of mRNAs. Until today, ongoing research deals with identifying dysregulated miRNAs that function, especially in cancer, as either tumor suppressors or oncogenes [78]. Overexpression and silencing experiments of ADAM8 demonstrated that ADAM8 and miR-720 positively correlated. The induction of miR-720 occurred in a non-catalytic manner dependent on integrin β-1/ERK1/2, thereby regulating invasion and metastasis [70]. However, seven oncogenic miRNAs were identified in blood analysis studies of PDAC patients. The abundance of ADAM8 in blood serum correlated with both disease progression, the presence of miR-451, and the absence of miR-720. It was also shown that the expression of both miR-451 and miR-720 depended on ADAM8 [79]. Additionally, ADAM8 was found to downregulate the expression of the tumor suppressor miR-181a-5p in GBM cell lines via activation of STAT3 and MAPK signaling. Consequently, MMP-9 expression and proliferation were increased in response to ADAM8-dependent downregulation of miR-181a-5p. Experiments in which functional ADAM8 mutants were introduced into ADAM8 knockout cells revealed cytoplasmic domain-dependent regulation of miR-181a-5p [65].

Essential functions of ADAM8 in intercellular communication

Both in homeostasis and the context of disease (inflammation, cancer, et cetera), all types of cells communicate by direct cell–cell contact, secretory proteins, or secretion of EVs [80]. Due to their size and origin, EVs can occur in three different forms: exosomes with a diameter of 30–150 nm, microvesicles with a diameter of 100–1000 nm, and apoptotic bodies with a diameter of 500–1000 nm. Exosomes differ from microvesicles and apoptotic bodies since secretion proceeds through the fusion of multivesicular bodies with the cell membrane [81]. The cargo of exosomes consists of proteins (for instance, transmembrane proteins and proteases), miRNAs, long-noncoding RNAs (lnc RNAs), mRNAs, DNA, lipids, and metabolites that accumulate during biogenesis [82]. However, both in inflammatory diseases as well as in cancer, exosomes play a vital role in cell–cell communication and disease progression [83,84]. In cancer, EVs mediate the communication between tumor cells and the TME, resulting in tumor progression, metastasis, and chemoresistance [83]. It was

demonstrated that ADAM8, secreted in exosomes, exerts its functions in the microenvironment of tumor and inflammation by either regulating the molecular content or acting as an active protease on the surface of the exosomes. The analysis of exosomes isolated from sera of control subjects, patients with precursor lesions, and PDAC patients exhibit the positive correlation of ADAM8 on exosomes with cancer progression. Evidence indicated that ADAM8 is embedded in the membrane of exosomes, and the extracellular domains are directed outward and proteolytic active [79,85]. Exosomes derived from both triple-negative breast cancer and PDAC cell lines exhibited an ADAM8-dependent secretion of LCN2. However, in exosomes derived from triple-negative breast cancer cells, ADAM8-dependent MMP-9 abundance was visible, but not for PDAC cells [30]. ADAM8 was detected on exosomes derived from GBM cell lines or serum of GBM patients [65]. As described above, ADAM8 is essential for the regulation of miRNAs, which are released into the circulation. One main delivery system of these miRNAs is exosomes, in which an ADAM8-dependent regulation of miR-451, miR-720, and miR-181a-5p secretion could be observed. miRNA-harboring exosomes could serve as a panel of potential biomarkers for the detection of precursor lesions, pancreatitis, PDAC, and GBM [65,79].

The intercellular communication mediated by ADAM8 is not only restricted to exosomes but further includes directly induced catalytic and non-catalytic events. The interaction of neutrophils with endothelial cells induces the expression of ADAM8 on the cell surface. This leads to the cleavage of adhesion molecules and binding of ADAM8 to integrins, altogether regulating the transmigration and recruitment of neutrophils to the inflammatory site [17,29,43,50]. Investigating the effects of ADAM8 knockdown on HB-EGFR downstream signaling in GBM revealed the diminished activation of EGFR, ERK1/2, and Akt. Additionally, less CCL2 was measurable, and fewer tumor-associated macrophages were recruited, postulating a positive feedback loop between tumor cells and macrophages that mediates chemoresistance [36,69]. Thus, as a jack of all trades, ADAM8 fulfills essential functions in intercellular communication in an autocrine, paracrine, and endocrine manner.

Future perspectives

Since its initial description in 1990, our knowledge on ADAM8 has significantly increased, and most studies report on ADAM8 as a relevant promoter of pathology when its expression is either upregulated by diverse inflammatory stimuli and inducers of cell stress such as chemotherapeutics or is constitutively high in tumor diseases. The low to no expression of ADAM8 under physiological conditions leads to the hypothesis that inhibition of ADAM8 as a treatment could have little to no side effects and may therefore be a reasonable drug target in diseases associated with immune cell recruitment, whether in inflammation or, similarly, in the tumor microenvironment. With the knowledge gained over the past decades, we can now clearly see that ADAM8 function is a combined effort of its different protein domains. This renders the manipulation of ADAM8 in the pathological context much more difficult to assess at different levels. Firstly, in malignancies, ADAM8 is expressed by multiple cell types within the tumor microenvironment in addition to the tumor localization, so that the targeting of distinct cell types should be considered to discriminate beneficial from detrimental effects. Secondly, the strategy of ADAM8 inhibition should be carefully considered, as the contribution of cleavage, disintegrin signaling, and intracellular SH3 signaling might vary in different pathological contexts. Thus, multi-domain inhibitors, as well as domain-specific inhibitors, are highly demanded to get a grip on ADAM8 in a therapeutic setting.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

LC and FGG performed a detailed analysis of the primary literature and prepared the data analysis and figures. LC, FGG, JWB, and DY wrote the draft of the manuscript and worked on the final version of this review. All authors read and approved the final version of the manuscript.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. The sample numbers used in Fig. 2B for each evaluation of normal versus tumor sample.
Table S2. The corresponding R and p-values of Fig. 3.
Table S3. Accession Numbers of transcript sequences used in Fig. 4A.
Table S4. Accession Numbers of protein sequences used in Fig. 4B and Fig. 5.
Table S5. The corresponding R and p-values of Fig. 6.
Table S6. The corresponding R and p-values of Fig. 7.