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REVIEW

# The pleiotropic roles of ADAM9 in the biology of solid tumors

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## Abstract

A disintegrin and a metalloprotease (ADAM) 9 is a metzincin cell-surface protease involved in several biological processes such as myogenesis, fertilization, cell migration, inflammatory response, proliferation, and cell–cell interactions. ADAM9 has been found over-expressed in several solid tumors entities such as glioma, melanoma, prostate cancer, pancreatic ductal adenocarcinoma, gastric, breast, lung, and liver cancers. Immunohistochemical analyses highlight ADAM9 expression by actual cancer cells and associate its abundant presence with clinicopathological features such as shortened overall survival, poor tumor grade, de-differentiation, therapy resistance, and metastasis formation. In each of these tumors, ADAM9 may contribute to tumor biology via proteolytic or non-proteolytic mechanisms. For example, in liver cancer, ADAM9 has been found to shed MHC class I polypeptide-related sequence A, contributing towards the evasion of tumor immunity. ADAM9 may also contribute to tumor biology in non-proteolytic ways probably through interaction with different integrins. For example, in melanoma, the interaction between ADAM9 and β1 integrins facilitates tumor stroma cross talks, which then promotes invasion and metastasis via the activation of MMP1 and MMP2. In breast cancer, the interaction between β1 integrins on endothelial cells and ADAM9 on tumor cells facilitate tumor cell extravasation and invasion to distant sites. This review summarizes the present knowledge on ADAM9 in solid cancers, and the different mechanisms which it employ to drive tumor progression.

**Keywords** ADAM9 · Solid tumors · Proteolytic · Non-proteolytic · β1 integrins

## Abbreviations

| | |
|---|---|
| ADAM9 | Disintegrin and metalloproteinase domain-containing protein 9 |
| MHC | Major histocompatibility complex |
| MMP1 | Matrix metalloprotease 1 |
| MMP2 | Matrix metalloprotease 2 |

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## Introduction

Members of a disintegrin and a metalloproteinase (ADAM) family are cell-surface endopeptidases most notably known for their role(s) in ectodomain shedding, cell proliferation, and cell adhesion [1, 2]. Structurally, a typical ADAM protein consists of sequentially conserved domains namely: a pro-domain, metalloprotease domain, a disintegrin domain, a cysteine-rich region, an EGF-like domain, a transmembrane domain, and a cytoplasmic domain [1–3]. The pro-domain region of ADAMs keeps the metalloprotease site inactive via a cysteine switch by keeping it in an inactive conformation [2, 4, 5]. The removal of this pro-domain either via autocatalysis or furin-type convertases switches the zinc coordination in the metalloprotease domain making it accessible for catalytic activity [2, 4]. Other studies show an alternative activation process of ADAM 10 and 17 that is independent of the removal of the pro-domain [6, 7]. These studies suggest that these two ADAMs adopt either a latent or an active conformation depending on the redox state of the cell environment [6, 7]. In ADAMs 10 and 17, the pro-domain is also required for proper intracellular trafficking

as it acts as a chaperone to ensure correct protein folding [5]. The disintegrin domain contains an integrin recognition motif responsible for binding and/or interaction with integrins [2, 3]. These interactions with different integrin families are known to regulate several biological activities including cell adhesion and cell–cell interactions [2, 3]. Several studies have, however, implicated this domain to a number of regulatory functions. For instance, in combination with the cysteine-rich region, they play a role in substrate targeting and can also facilitate the removal of the pro-domain to expose the catalytic domain [1–4].

Functionally, ADAMs have been linked to several biological processes including muscle development, cell–cell interactions, cell migration, sperm-egg interactions, axon guidance, immune functions, and determination of cell fate in the nervous system [1, 2]. A well-studied member of this family is ADAM17, also known as tumor necrosis factor-alpha converting enzyme (TACE) that orchestrates immune and inflammatory responses via proteolytic release of TNF-α or ligands of the epidermal growth factor receptor family [2]. In the context of cancer, several ADAMs including 8, 9, 12, 15, and 17 have been implicated in promoting malignant behavior [1–3]. Upregulation of ADAMs in human cancers often correlates with aggressive disease progression and poor clinical outcomes. This has attracted interest into the tumor-biological role(s) of ADAM proteases. The prognostic and diagnostic value of ADAM9 has been established in different solid tumors (Table 1). Based on its potential to shed several membrane-bound ligands capable of stimulating migration, adhesion, and proliferation, ADAM9 likely enhances malignancy. Reported substrates of ADAM9 include amyloid precursor protein (APP), heparin-binding epidermal growth factor (HB-EGF), collagen XVII, tumor necrosis factor-p75, fibronectin, fibroblast growth factor receptor 2, insulin B chain, and gelatin that have been implicated in various pathologies including cancer [1–3]. ADAM9 overexpression in solid tumors has been correlated with aggressive tumor phenotypes and unfavorable clinical prognosis [8–17]. Mechanistically, ADAM9 may drive tumor progression either via non-proteolytic or proteolytic mechanisms (Figs. 1 and 2]. Proteolytic mechanisms may involve shedding or processing of cell-surface proteins, for example CUB domain-containing protein 1 (CDCP1) or MHC class I polypeptide-related sequence A (MICA), which directly drive tumor growth [18]. On the other hand, non-proteolytic mechanisms include interactions between tumor cells and endothelial or peritumoral stromal cells mediated mainly by different integrin family members [19–21].

**Table 1** Summary of the effect of ADAM9 overexpression in different solid tumors gained from in vitro and in vivo experiments

| Cancer type | Effect of ADAM9 overexpression | References |
|---|---|---|
| Breast | Transendothelial cell migration<br>Adhesion to endothelial cells<br>Extravasation and invasion | [20, 38, 41] |
| Glioma | Poor tumor grade<br>Shortened overall survival<br>Proliferation, migration and invasion | [14, 43, 44] |
| Lung | Shortened overall survival<br>Invasion and brain metastasis<br>Proliferation and migration | [11, 46, 47, 51, 52] |
| Prostate | Epithelial–mesenchymal transition (EMT)<br>Therapy resistance<br>Proliferation and bone metastasis | [26, 53, 54] |
| Pancreas | Short overall survival<br>Poor differentiation<br>EMT, migration and invasion | [8, 23, 56, 57] |
| Gastric | Tumor-node and lymph-node metastasis<br>Proliferation and invasion<br>Cell adhesion | [9, 59, 60] |
| Liver | Poor differentiation<br>Intrahepatic metastasis and hepatic venous invasion<br>Immune evasion<br>Proliferation and resistance to apoptosis | [16, 18, 24, 61] |
| Melanoma | Cell adhesion<br>Tumor–stromal cell interactions<br>Invasion and metastasis | [17, 20, 63] |

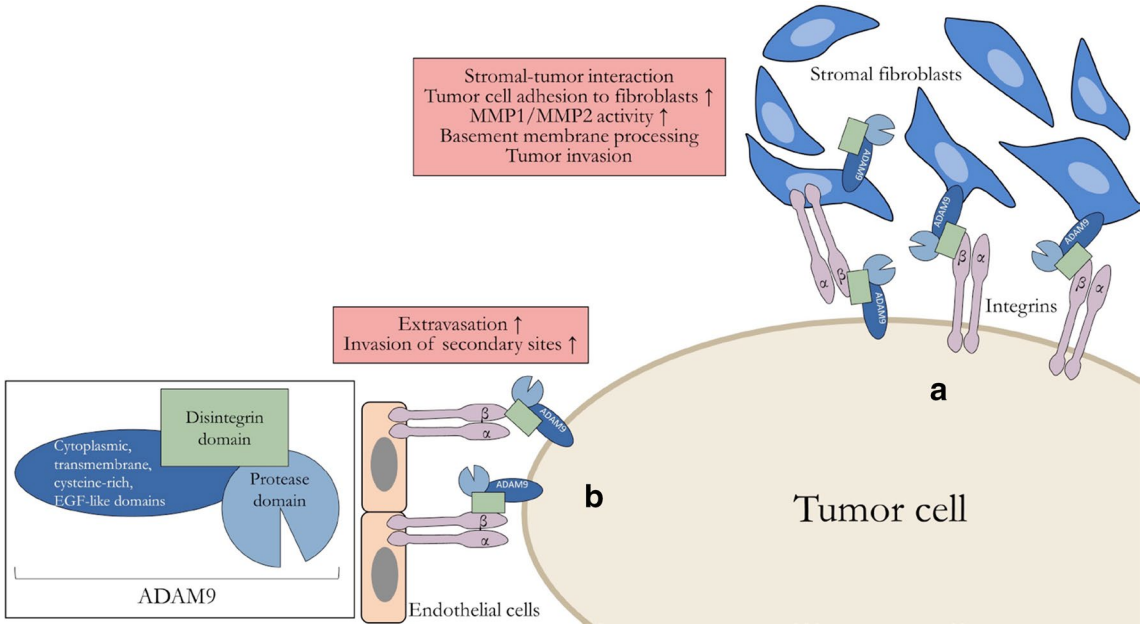


**Fig. 1** Non-proteolytic mechanisms of ADAM9-driven tumor progression. **a** Melanoma cells interaction with peritumoral stromal cells enhances tumor cell adhesion, which activates MMP1 and MMP2 leading to basement membrane processing and invasion. **b** Breast tumor cells interact with integrins on endothelial cells promoting tumor cell extravasation and invasion to distant sites

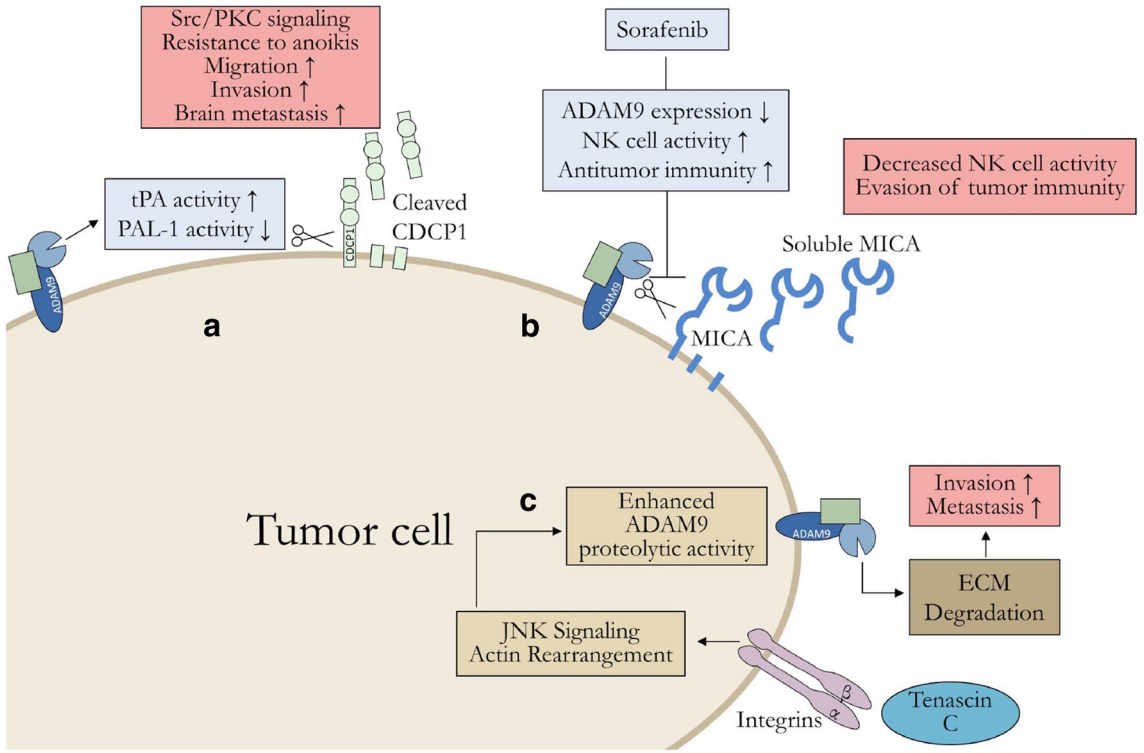


**Fig. 2** Proteolytic mechanisms of ADAM9-driven tumor progression (in different solid cancers). **a** ADAM9-mediated tPA shedding of CDPC1 enhances migration, invasion, and brain metastasis in lung cancer via Src and PKC signaling pathway. **b** ADAM9 shedding of MICA on the surface of hepatocellular carcinoma cells (HCC) leads to decreased NK cell activity and evasion of immunity, a phenotype that can be reversed by sorafenib (kinase inhibitor). **c** Tenascin-C-driven invasion of brain tumor-initiating cells (BTIC) that gives rise to glioma is characterized by increased ADAM9 proteolytic activity that degrades the extracellular matrix to promote migration and invasion

## ADAM9 regulation

In the context of tumor progression, ADAM9 expression is regulated by different factors including micro-RNAs and oxidative stress [9, 22–27]. Gene regulation by micro-RNAs is linked to their ability to bind to the 3′-untranslated region (3′-UTR) of target genes [22–25], which induces destabilization and decay of mRNAs suppressing translation. Expression profiling of tumor suppressive miRNAs shows that they are downregulated in tumors compared to adjacent normal tissues and associated with aggressive disease phenotype and poor prognosis [22–25]. ADAM9 is intensely regulated by different miRNAs with their expression profiles inversely correlating to each other. MiR-126 is an example of a micro-RNA that exerts its tumor suppressive effects by regulating the expression levels of ADAM9 in melanoma, osteosarcoma, pancreas, bladder, thyroid, and breast cancers [22, 23, 28–32]. Other tumor suppressive miRNAs shown to regulate ADAM9 expression are miR-203, miR-1274a, miR-1, miR-20b, miR-488, miR-543, and miR-154 in different solid tumors such as hepatocellular carcinoma, colon cancer, glioblastoma multiforme, breast, lung, and pancreatic cancers [24, 25, 33–37]. Oxidative stress is another key regulator of ADAM9 expression contributing towards cancer invasion and metastasis. Studies in different solid tumors including gastric, prostate, and lung cancers reveal a direct link between oxidative stress and ADAM9 expression [9, 26, 27]. Evidence from these studies shows that exposure of cancer cell lines to hypoxia, cell crowding, or hydrogen peroxide elevates ADAM9 expression, which correlates with increased migration and invasion. This phenotype is reversed upon the administration of antioxidants or hydrogen peroxide degrading enzymes. These results point to reactive oxygen species (ROS), generated by cells under conditions of stress, as a mediator that regulates ADAM9 expression [9, 26, 27]. The present review will highlight the current knowledge on ADAM9 with a major focus on its role in the progression of solid cancers (Figs. 1, 2).

## Breast cancer

Breast cancer is one of the solid tumors for which multiple reports suggest that ADAM9 contributes significantly to disease progression [15, 19, 38–41]. O'Shea et al. presented the initial study that investigated the mRNA and protein expression levels of ADAM9 in infiltrating ductal/lobular breast carcinomas compared to normal breast tissue [15]. Their results showed that ADAM9 mRNA was frequently expressed in breast carcinomas (72/110, 66%) compared to normal breast tissues (6/25, 24%). The 84 kDa form of the protease correlated positively with node-positive cancers ($p = 0.05$) and HER-2 protein levels ($p = 0.016$) [15]. Micocci et al. demonstrated using *MDA-MB-231* invasive ductal breast cancer cells that RNAi silencing of ADAM9 inhibited tumor invasion in vitro without affecting proliferation and migration [38]. This supports the hypothesis that ADAM9 may play a role in invasion and metastasis of breast cancer. Years later, Micocci et al. justified this observation via a transendothelial cell migration experiment using *MDA-MB-231* cells. RNAi silencing of ADAM9 in these cancers cells strongly inhibited their migration through HUVEC (50%), HMEC-1 (40%), and HMVEC-Der-dLy cells (32%) compared to controls [19]. These results substantiated that ADAM9 participates in MDA-MB-231 extravasation, a process that involves migration via the vasculature, adhering to endothelial cells, extravasation, and ultimately invasion into secondary tissues [19].

Moelans et al. did a molecular profiling of invasive breast cancer samples to determine the genes that drive progression and resistance to therapy [39]. Using the multiplex ligation-dependent probe amplification (MLPA), they analyzed 20 breast tumor-related genes in 104 patient samples. ADAM9 gene amplification was identified in 32% of the samples and positively correlated with the tumor mitotic index, histological grade, and estrogen receptor status [39]. Fry and Toker adopted a different approach towards investigating ADAM9 biology in breast cancer. The authors showed that two alternative spliced variants of ADAM9 (ADAM9-L-transmembrane protease and ADAM9-S-secreted variant) have opposing effects on breast cancer cell migration in BT549 breast cancer cells [39]. ADAM9-S promoted cell migration in a metalloprotease-dependent way, while ADAM9-L suppressed cancer cell migration in a disintegrin domain-integrin binding manner but independent of its proteolytic activity [40]. This opposite phenotypes may be related to the localization of these two variants as ADAM9-S is able to process certain substrates or the extracellular matrix not accessible to ADAM9-L due to membrane tethering. As both isoforms are expressed in breast cancer cells, their relative levels act as key determinants in the determination of aggressive breast cancer phenotypes [39]. These results were confirmed by Mazzocca et al. who showed that the secreted ADAM9 (ADAM9-S) by hepatic stellate cells promoted cell invasion in different human tumor cell lines in matrigel assays [41]. This invasive phenotype was dependent on the protease activity and integrin interactions. Analysis of liver metastases revealed that ADAM9 is expressed by stromal myofibroblasts localized at the tumor invasive front and these tumor–stromal interactions drive invasion and colonization of the liver [41].

## Glioma

There is increasing evidence that ADAM9 overexpression plays a significant role in the progression of gliomas. Expression profiling by The Cancer Genome Atlas (TCGA) highlighted that elevated levels of ADAM9 correlate with shortened survival in glioblastoma multiforme (GBM). Fan et al. found that ADAM9 mRNA expression was higher in GBM than in lower grade gliomas (LGG) patients [14]. Furthermore, among LGG patients, the aggressive astrocytic cancers showed high ADAM9 mRNA expression, which correlated with histological type, tumor grade, poor clinical outcome, and shortened overall survival [14]. Tenascin-C, a major extracellular matrix protein of malignant gliomas, has been shown in vitro to stimulate the invasion of glioma cell lines U87 and U251 [42]. However, its regulatory role on brain tumor-initiating cells (BTIC) that give rise to glioma was not well understood. Using a range of in vitro assays including microarray analysis and RNA interference, Sarkar et al. showed that tenascin-C promoted the invasion of BTICs in a metalloprotease-dependent way, a phenotype that was abolished upon administration of protease inhibitor BB94 [43]. Microarray analysis of gene expression revealed ADAM9 as a potential regulator of this invasiveness, a finding corroborated by increased ADAM9 protein expression in four patient-derived BTIC cell lines [43]. Moreover, the implantation of BTICs xenografts in mice revealed an overexpression of ADAM9 at the invasive front compared to the tumor mass suggesting a role of ADAM9 in cell migration and invasion. Silencing of ADAM9 expression, as well as inhibiting the mitogen-activated protein kinase-8 (MAPK8), inhibited TNC-stimulated ADAM9 expression, proteolytic activity, and BTIC invasion [43]. These results suggest that TNC drives BTIC invasion by enhancing the proteolytic activity of ADAM9 in an MAPK8-dependent manner. These results were validated using immunohistochemistry on resected human glioblastoma samples and orthotopic xenografts that showed increased TNC and ADAM9 expression at the tumor invasive fronts [42, 43].

Further supporting this view, Liu et al. described the tumor suppressive role of miR-140 in glioma through its direct targeting of ADAM9 [44]. Their findings showed that miR-140 was downregulated in glioma tissues and cell lines and this correlated with WHO grading of gliomas. Restoration of miR-140 expression in glioma cell lines attenuated cell proliferation, migration, and invasion by directly targeting ADAM9. These results were confirmed by RNAi of ADAM9 which amplified the tumor suppressive functions of miR-140, while overexpression of ADAM9 abrogated these suppressive effects [44]. The tumor-promoting role of ADAM9 in gliomas was corroborated by Formolo et al. who used proteomics to investigate the secretome signature of four glioblastoma cell lines (U87, U118, T98, and LN18) [45]. The four cell lines differ in invasion capacities with U87 being the most invasive, which may suggest additional secreted proteins that contribute to this aggressive phenotype. They identified several proteins that were exclusively or highly expressed by U87 cells compared to the least invasive GBM cells including the proteolytic enzymes cathepsin B, ADAM9, and ADAM10 which are known to promote invasion in gliomas [45].

## Lung cancer

Findings from lung cancer studies demonstrate that ADAM9 overexpression is a major player in the progression of lung cancer and regulation of metastasis. The earliest in vitro study on ADAMs in lung cancer by Shintani et al. investigated the relationship between its expression and brain metastasis. The authors used a sub-line of a lung cancer cell line (EBC-1), which are highly metastatic to either the bone or the brain [11]. ADAM9 mRNA levels were significantly higher in the brain-metastatic sublines than in bone-metastatic and parental lines. Stable transfection of full-length ADAM9 protein in A549 and EBC-1 cells resulted in increased invasion, increased adhesion to brain tissues, and overexpression of α3 β1 integrins. The increased invasion and adhesion to the brain was attenuated by the administration of an anti-β1 blocking monoclonal antibody suggesting a likely pathway of ADAM9 mediated brain metastasis [11]. These results were validated in vivo following the administration of A549 overexpressing ADAM9 and mock controls. The former developed lung cancer with micro metastasis in the brain, while mock controls developed lung tumors without brain metastasis [11]. Zhang et al. investigated the abnormal expression of ADAM9 in human resected non-small cell lung cancer using immunohistochemistry and correlated its levels with clinicopathological features [46, 47]. ADAM9 was highly expressed in tumors compared to normal lung tissues, and non-small cell lung cancer (NSCLC) patients with high ADAM9 levels had shortened 5-year survival rates compared to those expressing low ADAM9 levels (56.9 vs. 88.9%, respectively). Multivariate analysis also showed that overexpression of ADAM9 was an independent factor of shortened survival time outlining its role in NSCLC pathology [46, 47].

The overexpression studies mentioned above were validated by Chang et al. using a loss-of-function system in A549, an NSCLC cell line followed by analysis of proliferation, apoptosis, invasion, and migration [48]. Downregulation of ADAM9 using shRNAs in A549 cells attenuated

migration, proliferation, invasion, as well as apoptosis in vitro. In vivo tumor growth was also suppressed in lung metastasis mouse models [48]. ADAM9-mediated brain metastasis was described in detail much later and is dependent on the plasminogen activator pathway. Lin et al. demonstrated that ADAM9 enhanced the ability of tissue plasminogen activator to cleave CDCP1, a promigratory protein that promotes metastasis to the brain [49]. An analysis of clinical specimens showed that high levels of ADAM9 correlated with high levels of CDCP1 and this contributed to poor clinical prognosis and high mortality risk [49, 50]. These results were further supported by the results of Chiu et al. who showed that enhanced CDCP1 expression and activity correlated with decreased expression of miR-128, a known tumor suppressor lost in cancer specimens [50]. While high ADAM9 expression correlates with metastasis to the brain as demonstrated by these authors, the mechanism of action remains unclear. Follow-up studies by the revealed that angiogenesis is a crucial step that drives metastasis to the brain [51]. Microarray analysis of ADAM9-regulated genes upon vascular remodeling showed that angiopoietin-2 (ANGPT2), tissue plasminogen activator (PLAT), and vascular endothelial growth factor A (VEGFA) expression were decreased in ADAM9-silenced cells. This in turn attenuated angiogenesis and tumor growth in vivo [51]. Immunohistochemical analysis showed that high expression of ADAM9, VEGFA, and ANGPT2 correlated with poor prognosis in lung adenocarcinoma patients. Together, these results explain how ADAM9 drive metastasis by modulation of vascular remodeling and angiogenesis [51]. Kossmann et al. also demonstrated that ADAM9 drives metastasis in aggressive lung adenocarcinoma in an angiogenesis-dependent mechanism. Intravenous injection of A549 cells with stable downregulation of ADAM9 (shADAM9) in nude mice showed reduced number of nodules in the lungs suggesting a lowered ability to extravasate and metastasize [52]. Subcutaneous injection of these cells in nude mice resulted in smaller tumors with a few neovessels compared to controls. These results were validated in vitro where tube formation in human umbilical vein endothelial cells (HUVEC) treated with supernatants from A549 shADAM9 cells was significantly reduced compared to controls [52]. Using a human antibody array mechanism, Kossmann et al. revealed the underlying molecular mechanism that involved interleukin 8 and CXCR2 interaction that promote angiogenesis both in vitro and in vivo as both cytokines were decreased in shADAM9 A549 cells emphasizing the critical role of ADAM9 in driving this aggressive lung adenocarcinoma phenotype [52].

## Prostate cancer

Clinical studies of prostate cancer correlate the expression of ADAM9 to the prognosis of the disease. Fritzsche et al. demonstrated this in a cohort of 198 samples where ADAM9 mRNA and protein levels were higher in tumors compared to normal tissue and strongly associated with PSA relapse-free survival [13]. Microarray analysis of prostate cancer cell line, LNCaP, revealed elevated levels of ADAM9 as the cells transition from an androgen-dependent to androgen-independent state [26]. This elevated level of ADAM9 was also observed in malignant prostate tissues compared to benign glands suggesting a role prostate cancer progression. Sung et al. showed that ADAM9 mRNA and protein levels in prostate cancer cells (LNCaP and C4-2) were increased upon exposure to stressful condition such as hydrogen peroxide, hypoxia, and cell crowding [26]. This induction, at least at the protein level, was attenuated upon the administration of an antioxidant ebselen or the expression of catalase, a hydrogen peroxide degrading enzyme in both cell lines. These results point to reactive oxygen species (ROS), generated by cells under conditions of stress, as a mediator that enhances ADAM9 expression [26].

Josson et al. demonstrated that ADAM9 contributes towards prostate cancer progression and might be a therapeutic target. The authors showed that ADAM9 expression induces alterations in the epithelial phenotype via modulation of integrin and E-cadherin expression [53]. Loss-of-function experiments using the C4-2 prostate cancer cell line resulted in sensitization to chemo/radiotherapy suggesting a role of ADAM9 in therapy resistance [53]. Liu et al. showed both in vitro and in vivo that ADAM9 might be a drug target for the treatment of prostate cancer. Short hairpin RNAs against ADAM9 inhibited the proliferation of prostate cancer cells (PC3 and LNCaP) as well as blocking growth of tumors and bone metastasis in murine models [54]. The decrease in proliferation was due to G1 arrest and a decrease in the population of cells in the S-phase. Microarray studies revealed decreased levels of REG4 in ADAM9 knockdown cells, which coincided with an increase in p21 that blocks the G1/S transition [54]. Pen et al. also evaluated the combination of urinary VEGF levels and tissue ADAM9 expression in patients with late-stage prostate cancer as markers of lethal phenotypic cancer [55]. The authors found that increased ADAM9 nuclear staining in relapsed tumors correlated with transition to the lethal phenotype. This was based on immunohistochemical evidence of elevated ADAM9 levels in the high-grade tumor regions of patients treated with hormone therapy. However, why ADAM9 localizes to the nucleus and the role which it plays remains to be elucidated. They

suggested that ADAM9 in combination urinary VEGF may serve as vital markers for phenotypic prediction of the risk of relapse following hormone therapy [55].

## Pancreatic cancer

Gene expression studies showed elevated levels of ADAM9 in patients with pancreatic ductal adenocarcinoma (PDAC) as demonstrated by Grützmann et al. using immunohistochemistry. The study used 59 PDAC samples, 11, endocrine tumors, 32 chronic pancreatitis, and 24 acinar cell carcinoma samples with ADAM9 staining observed in 58/59 PDACs, 2/24 acinar carcinomas and 0/32 endocrine tumors [8]. These results open the possibility to use ADAM9 expression to differentiate PDAC from other solid pancreatic tumors. In PDAC samples, cytoplasmic expression of ADAM9 strongly correlated with shorter overall survival and poor differentiation than in cases of apical membrane expression ($p=0.001$) [8]. Using an Affymetrix cancer array, Alldinger et al. analyzed the gene expression of five established PDAC cell lines (AsPC-1, BxPC-3, Capan-1, Capan-2, and HPAF-II) and five primary isolates, one of them from benign duct cells [56]. Gene expression analysis from three replicate experiments revealed 122 upregulated genes in the tumor cells with ADAM9 among those with a fold change greater than 3 [56]. The same results were obtained in a study by Yamada et al. who quantified the expression of ADAM9 in microdissected samples of 11 cancer cells and 11 normal pancreatic epithelial cells. The results showed that the cancer cells expressed higher levels of ADAM9 compared to the normal epithelial cells ($p=0.016$) [57].

Hamada et al. investigated the tumor suppressive role of micro-RNAs in pancreatic cancer and the underlying molecular mechanisms. A comprehensive analysis of micro-RNA expression in PDAC tissues revealed the reduced expression of miR-126 compared to normal pancreas tissues [23]. Re-expression of miR-126 in pancreatic cancer cells reduced the protein expression levels of ADAM9 and inhibited migration and invasion in AsPC-1 and Panc-1 cells. Re-expression of miR-126 also increased the expression of E-cadherin with a concomitant decrease in N-cadherin rendering the cells more epithelial [23]. miR-126 exerts its tumor suppressive effects by directly binding to the 3′ untranslated region of ADAM9, thus, interfering with the expression of the protease. Similarly, Van Kampen et al. identified miR-520f as a novel tumor suppressor gene in pancreatic cancer, which upon re-expression reverses epithelial-to-mesenchymal transition (EMT) [58]. This reversal of EMT is associated with increased E-cadherin expression, attenuated migration, and invasion in multiple cell line models. Using the 3′-UTR assay, the authors identified ADAM9 as a direct target of miR-520f suggesting a role of this protease in driving metastasis in pancreatic cancer [58].

## Gastric cancer

A number of studies have also linked the expression of ADAM9 to poor prognosis in gastric cancer. Carl-McGrath et al. investigated the pathogenesis of different ADAMs, namely 9, 12, and 15, using qPCR and immunohistochemistry in cancer tissues and corresponding non-tumor tissues as well as five gastric cancer cell lines [59]. Together with other ADAMs, ADAM9 was significantly upregulated in tumor tissues compared to normal gastric tissues. Administration of anti-ADAM9 antibody inhibited cell growth in vitro in gastric cancer cells suggesting a role of ADAM9 in cell proliferation either via shedding of signaling molecules or interaction with adhesion molecules [59]. Wang et al. reported an aberrant expression of ADAM9 in gastric cancer tissues which correlated with different clinicopathological features such as local invasion, tumor size, lymph node metastasis, and tumor-node metastasis [60]. Knockdown of ADAM9 in a GC cell expressing the highest levels of ADAM9 attenuated cell proliferation, which coincided with cell cycle arrest at the G0/G1 phase [59]. Moreover, they found that ADAM9 expression was attenuated in GC cells following the re-expression of miR-126 coinciding with studies done in other solid tumors [23, 28–31].

Kim et al. demonstrated that ADAM9 is a key player in gastric cancer progression and that this was dependent on its protease activity. Inhibition of ADAM9 activity using RAV-18 (an ADAM9 blocking antibody) showed a dose-dependent decrease of enzyme activity only in high ADAM9 expressing GC cells [9]. A dose-dependent decrease in proliferation and invasion was also observed in high ADAM9 activity GC cells unlike low ADAM9 activity GC cells where no significant effect was observed [9]. Exposing the low ADAM9 activity cells to hypoxia increased ADAM9 activity and subsequent invasion which was abrogated upon the administration of RAV-18. Immunoblot analysis under hypoxic conditions confirmed that increased invasiveness of these cell lines correlated with increased ADAM9 protein expression suggesting a link between hypoxia and ADAM9 expression [9]. Silencing of HIF-1α under hypoxia suppressed ADAM9 protein expression, protease activity, as well as reduction of EGFR and ERK activities. These results were confirmed in xenograft models where treatment of mice with RAV-18 decreased tumor growth compared to the control group [9]. This inhibition of ADAM9 in gastric cancer using anti-ADAM9 antibody RAV-18 suggests a potential therapeutic effect that could be exploited in treating gastric cancer and possibly several solid tumors. However, it has to be noted the use of RAV-18 as a potential

therapeutic effect is dependent on ADAM9 expression levels, activity, subcellular localization of the protease, and in tumors where ADAM9 expression increases in response to hypoxia, at least based on the results of this study [9]. The anti-tumor potential of RAV-18 remains to be fully exploited in the treatment of other solid tumors, especially those under ADAM9-mediated progression.

## Liver cancer

There is evidence suggesting that the overexpression of ADAM9 is correlated to clinicopathological features in hepatocellular carcinoma and may contribute to poor prognosis [16, 18, 61]. Kohga et al. investigated the pharmacological effects of sorafenib in HCC cells and identified ADAM9 as a major sheddase of major histocompatibility complex class I-related chain A (MICA) [18]. Knockdown of ADAM9 in HCC cells resulted in increased expression of MICA on the cell surfaces and decreased abundance in the culture supernatant. These results were confirmed by introducing mutations at ADAM9 cleavage sites on MICA that led to less shedding of the protein. Treatment of HCC cells with sorafenib increased the expression of membrane-bound MICA by decreasing ADAM9 expression levels [18]. Tao et al. investigated the prognostic value of ADAM9 by comparing the survival of patients with and without ADAM9 expression. ADAM9-positive patients had larger and poorly differentiated tumors, more intrahepatic metastasis and bile duct and hepatic venous invasions than ADAM9 negative patients [16]. These results were also reflected in a protein microarray study done by Tannapfel et al. to determine the protein profile of hepatocellular carcinomas [61]. The authors quantified the protein levels of 83 different proteins in normal liver and HCC tissues and corroborated their findings using western blots. The microarray approach revealed several proteins upregulated in HCCs and ADAM9 was one of the proteins identified [61].

Li et al. evaluated the importance of ADAM9 signaling in response to liver injury induced by carbon tetrachloride ($CCl_4$) in vivo [62]. Mice treated with anti-ADAM9 monoclonal antibody (Anti-ADAM9 mAb) following $CCl_4$ injection displayed aggravated liver injury, which attenuated interleukin-6 trans-signaling and downregulation of phospho-STAT3, VEGF, and proliferating cell nuclear antigen (PCNA) compared to control group mice [62]. IL-6 trans-signaling via pSTAT3 and VEGF is an important modulator of angiogenesis in acute liver injury and plays a role in repair pathways. Pre-treatment of mice with recombinant ADAM9 reversed the impact of anti-ADAM9 mAb suggesting a role of ADAM9 in angiogenesis and hepatocyte proliferation in liver injury repair [62]. A number of micro-RNAs have been identified as ADAM9 regulators and potential therapeutic strategies [24, 25]. Wan et al. reported that the downregulation of miR-203 in HCC correlated with shortened patient survival [24]. Re-expression of this micro-RNA resulted in decreased expression of ADAM9 in a post-transcriptional regulatory way, inducing apoptosis and decreasing proliferation and invasion [24]. Zhou et al. evaluated the miRNA profile in HCC cells in the presence or absence or sorafenib. Treated cells showed an upregulation of miR-1274a that repressed the expression of ADAM9, a likely anti-tumor mechanism of sorafenib in HCC [25].

## Melanoma

ADAM9 overexpression has also been associated with poor prognosis in melanoma patients. Zigrino et al. analyzed the protein expression of ADAM9 in melanoma samples and showed that it is restricted within the tumor invasive front [17]. Interestingly, they also detected ADAM9 in the peritumoral stromal fibroblasts but not in the fibroblasts far away from the tumor site. This observation may suggest a sort of paracrine communication between the melanoma cells and stromal fibroblasts adjacent to the tumor site, thereby driving tumor progression [17]. A follow-up study in 2011 by Zigrino et al. showed that ADAM9 expression is crucial in facilitating the interaction between melanoma cells and peritumoral stromal fibroblasts, which contributes towards melanoma invasion [20]. Using a recombinant disintegrin-like and cysteine-rich domain of ADAM9 (DC-9), they showed that fibroblasts and melanoma cells adhered to immobilized DC-9 in an $Mn^{2+}$-dependent way, a process mediated by β1 integrins. This interaction also led to increased expression of MMP-1 and MMP-2 explaining the high invasive capacity [17, 20]. These results were validated by Mygind et al. [21], who showed that migration and adhesion of PC3 cells were dependent on the interactions between ADAM9 and β1 integrins.

Using a melanoma mouse model, Giebeler et al. showed that deletion of ADAM9 in HGF/Cdk4 mice impaired melanoma development as well as metastasis. They knocked out ADAM9 in mice with HGF transgene and $Cdk4^{R24C/R24C}$ knock-in mutation and used these mice to study the function of ADAM9 in stromal and melanoma cells [63]. There was less spontaneous tumor development and lung metastases in $ADAM9^{-/-}$ mice compared to control mice. Mechanistically, they used in vitro assays to understand these effects and showed that ADAM9 deletion in melanoma cells reduced adhesion and transmigration to/through the endothelium [63]. This implies that ADAM9 mediates extravasation of melanoma cells in a cell-autonomous manner. They also demonstrated that laminin β3-chain, a component of basement membrane, is a direct substrate of ADAM9 and its processing leads to basement membrane disruption facilitating

tumor invasion. ADAM9 knock out decreased the invasion of melanoma cells in the basement membrane, a phenotype that was reversed upon administration of soluble ADAM9 [63].

## Consensus and conclusion

For many entities of solid tumors, there are multiple studies linking ADAM9 to tumor progression, metastasis formation, and/or worsened outcome. Mechanistically, the details remain unclear, but it is highly likely that both proteolytic and non-proteolytic properties contribute to these observed effects. For instance, metastases of tumors such as glioma and lung cancer are dependent on ADAM9 metalloprotease activity, which promotes extracellular matrix degradation and shedding of pro-invasion molecules, respectively [42, 43, 48, 49]. On the other hand, in breast cancer and melanoma, interaction between ADAM9 and various integrins has been shown to promote cancer cell extravasation and subsequent invasion to distant sites [19, 20]. A clear consensus that emerges in this review is that ADAM9 overexpression promotes tumor growth, invasion, and metastasis using different mechanisms depending on the type of solid cancer. In terms of regulation, ADAM9 expression is regulated by a number of micro-RNAs with anti-tumor effects with miR-126 extensively studied in different solid tumors. These micro-RNAs regulate ADAM9 by binding to the complementary 3′-UTR sequence destabilizing the mRNA and attenuating translation [22–25]. Several studies showed that the re-expression of these micro-RNAs in cancer cell lines arrested tumor progression by inhibition of proliferation, invasion, migration, and vascular formation in vitro [22]. This direct regulation of ADAM9 by micro-RNAs creates a potential niche for micro-RNA-based therapy development to be used in clinical management of tumors. Oxidative stress introduced by hydrogen peroxide, cell crowding, and hypoxia has been shown to regulate ADAM9 expression [9, 26, 27]. The common mediator under these stress condition is intracellular ROS that enhances ADAM9 transcription and subsequent protein translation. Quenching of this oxidative stress either via use of antioxidants or re-expressing of catalase attenuates oxidative stress-induced ADAM9 expression and cancer progression in vitro [26, 27].

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