ADAMs and ADAMTSs in cancer

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ABSTRACT

ADAMs and ADAMTSs are multi-domain proteins characterised by the presence of both metalloproteinase and disintegrin-like domains. ADAM proteins are usually type 1 transmembrane proteins, and ADAMTSs are secreted from cells. The dysregulated expression of ADAMs and ADAMTSs has been reported in a wide range of human cancers, where, in many cases, they are implicated as positive regulators of cancer progression. Proteolytically active ADAMs act as ectodomain sheddases, which release extracellular regions of membrane-bound proteins (e.g., adhesion molecules, growth factors, cytokines, chemokines and receptors). Certain ADAMTSs break down extracellular matrix (ECM) proteoglycans (e.g., aggrecan, brevican and versican). Through these actions they are able to sculpt the tumour microenvironment and modulate key processes involved in cancer progression, including cell proliferation, migration and angiogenesis. Members of both groups of protein can also act to inhibit or slow cancer progression: ADAMs can interact with specific integrins to elicit inhibitory effects on cancer dissemination, and certain ADAMTSs possess anti-angiogenic activity, which prevents an increase in tumour size. This review covers recent developments in the involvement of ADAM and ADAMTS proteins in human cancer.

KEY WORDS: ADAM proteins.
ADAMTS proteins, human.
Neoplasm metastasis.
Neoplasms.

Introduction

With over 10.9 million new cases of cancer diagnosed per annum, 6.7 million deaths and 24.6 million persons living worldwide with cancer,[1] understanding the intricate nature of this disease is more imperative than ever before.

The progression from a benign non-invasive tumour to a malignant neoplasm capable of dissemination throughout the host is a complex multistep process. The hallmarks of cancer cells include the ability to proliferate independently of growth/antigrowth signals, to stimulate sustained angiogenesis, to degrade the surrounding extracellular matrix (ECM), to modulate cellular adhesion and migration capabilities, and evade apoptosis.[2,3]

The destructive processes involved in cell invasion and metastasis are not exclusive to cancer progression but occur routinely during wound repair, vasculogenesis and axon outgrowth.[4] However, these processes are less controlled in cancer cells. Invading malignant cells interact with basement membranes or the ECM in order to advance. This involves three key processes: i) attachment of malignant cells to the matrix, ii) proteolytic breakdown of the ECM, and iii) migration of invading cells through the damaged ECM.[5] The proteolysis events orchestrating the destructive process of normal and non-malignant pathologies are controlled and self-limiting; those involved in tumour invasion appear to occur perpetually, with a loss of controlling mechanisms, which can result in the formation of secondary tumour.[4,6]

Malignant cells cross the basement membrane at least three times during metastasis – to escape their primary site, infiltrate the vascular system, and then extravasate from the bloodstream into a target organ.[5,6] This is mediated by a number of different proteolytic enzymes, which could be released from the invading tumour,[6] stromal fibroblasts surrounding the tumour and localised inflammatory cells (macrophages and neutrophils).[7] The highest activity levels of proteases involved in cancer dissemination are found at the invading front of the tumour where degradation of normal tissue is occurring.[6]

Currently, proteinases are divided into five classes: metallo-, serine, aspartate, cysteine and threonine proteinases; with the dysregulated expression of members of each proteinase class being implicated in tumour invasion and metastasis.[8] As the roles of the primary ECM remodelling enzymes, the matrix metalloproteinase (MMPs, zinc-dependent proteinases), have been reviewed extensively,[9,10] they will not be assessed here. Due to the identification of dysregulated expression in a wide range of cancer types, this review will focus on the possible roles of the 'a disintegrin and metalloproteinase' (ADAM, Table 1) and the 'a disintegrin and metalloproteinase with thrombospondin motifs' (ADAMTS, Table 2) proteins in cancer progression.

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A disintegrin and metalloproteinase (ADAM) proteins

ADAMs are multi-domain transmembrane proteins, forming one of four distinct subfamilies of the metzincin zinc-dependent protease superfamily, the adamalysins.[11] They are expressed in a wide range of animal species, tissues and cell types, and have been implicated in sperm-egg fusion, spermatogenesis, neutrophil infiltration, platelet aggregation, neurogenesis and cachexia.[12]

Over 29 ADAM proteins have been identified in humans to date,[13] and these can be grouped broadly according to

their distribution and functions. The first group, termed the 'ectodomain sheddases,[14] encompasses ADAMs that are distributed throughout the body and have an active metalloproteinase domain (ADAMs 1, 8, 9, 10, 12, 15, 17, 19, 28 and 33). These enzymes are involved in the proteolysis of the ectodomains of membrane-anchored cytokines, growth factors and their receptors,[12,15] allowing cells to alter responsiveness to their environment. The second group (ADAMs 2, 3, 11, 22 and 23) are predicted to have an inactive metalloproteinase domain, effectively limiting their function to adhesion/de-adhesion and cell fusion.

The third group contains 15 ADAM proteins (ADAMs 2, 3, 5, 6, 7, 16, 18, 20, 21, 24, 25, 26, 29, 30 and 32), which are expressed exclusively in the male gonads (testis and epidermis), where some have a role in sperm maturation.[16] Of these, ADAMs 20, 21 and 30 have known proteolytic activity and ADAMs 2, 3, 7 and 32 have a predicted inactive metalloproteinase domain.

ADAM domain structure

ADAM proteins are approximately 750 amino acids in length and have a characteristic seven-domain structure (Fig. 1) comprising the prodomain, metalloproteinase, disintegrin-like, cysteine-rich, EGF-like, transmembrane and cytoplasmic tail domains.[17] In addition, ADAM proteins are synthesised with an N-terminal signal peptide to direct them into the secretory pathway.[18]

All ADAM proteins are synthesised as zymogens with the approximate 200 amino acid prodomain located at their N-terminus, which acts to maintain the latent, inactive state of the immature protein.[18] Activation of ADAM zymogens is facilitated in the trans-Golgi network by furin-like proprotein convertases, which remove the prodomain at a furin recognition site (RxxR sequence; the single-letter amino acid code x is for any other amino acid), located between the prodomain and the metalloproteinase domain.[19] This results in the formation of a mature-length protein with an unobstructed, active catalytic site.[20] Although most ADAM proteins are thought to be activated by this process, notable exceptions are ADAM8 and ADAM28, which can undergo autocatalytic activation.[21,22] The prodomain has a secondary function in the proper folding of ADAM proteins, in particular the metalloproteinase domain, as ADAM proteins synthesised without a prodomain are proteolytically inactive.[23–25] The prodomain is also necessary for proper transit of ADAM proteins through the secretory pathway, as a form of ADAM12-S lacking the prodomain is retained in the early endomembrane system.[18,23]

The metalloproteinase domain of ADAM proteins (~200 amino acids) contains the active site consensus sequence HExxHxxGxxHD.[26] A tetrahedral coordination sphere is formed by the three histidine residues binding an essential zinc ion, and the glutamic acid residue acting as a catalytic support for the required water molecule.[27] The glycine residue allows a turn in the peptide backbone, which, together with an essential downstream methionine residue located in a Met turn motif,[18] completes the active site

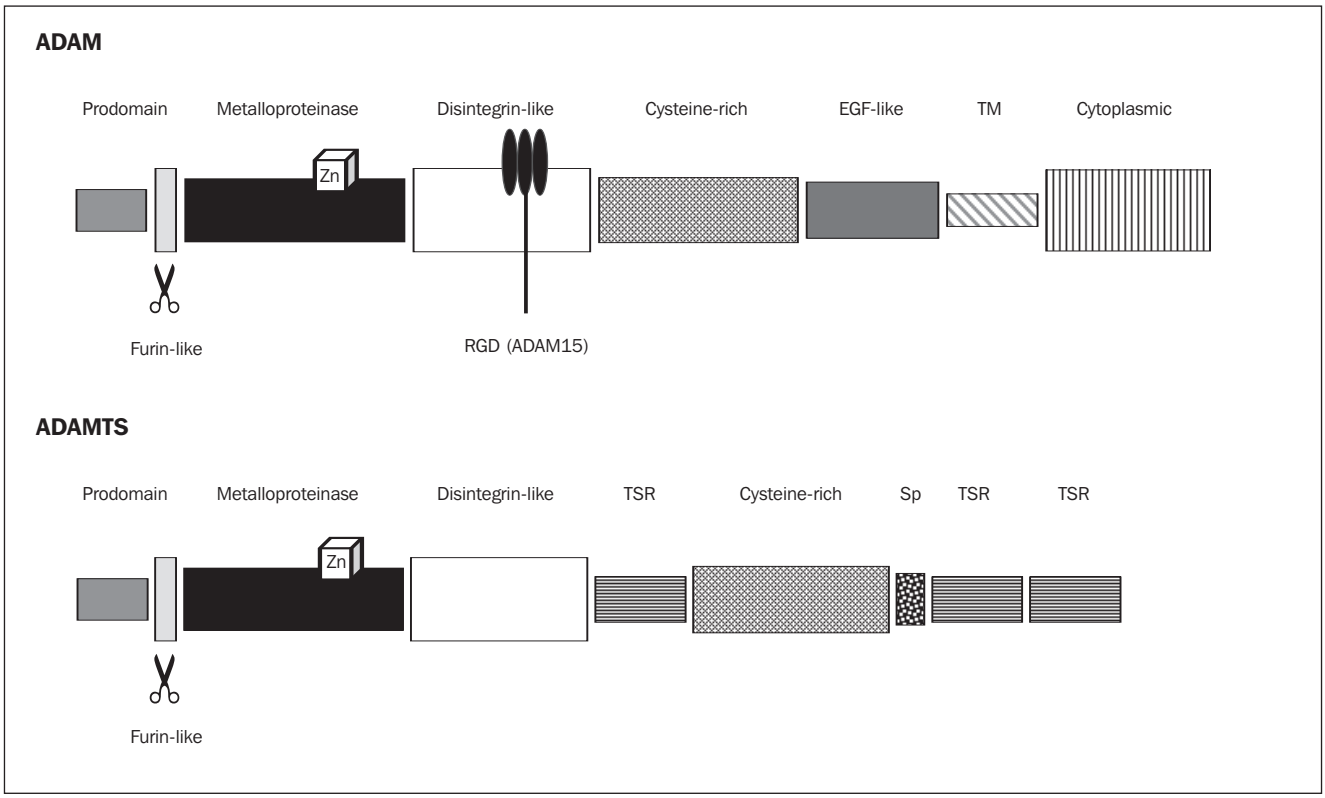

Fig. 1. Domain structures of ADAM and ADAMTS proteinases. ADAM proteins consist of seven common domains – prodomain, metalloproteinase, disintegrin-like, cysteine-rich, EGF-like, transmembrane (TM) and cytoplasmic tail domains. ADAM15 is the only ADAM protein with the RGD motif characteristic of true disintegrin proteins. ADAMTS proteins also contain the prodomain, metalloproteinase, disintegrin-like and cysteine-rich domains, but the remaining domains are composed of variable numbers of thrombospondin repeats (TSRs) and a spacer domain (Sp). They may also contain additional domains unique to individual ADAMTSs (e.g., gon, CUB [not shown]). Most ADAM and ADAMTS zymogens are processed by furin-like proprotein convertases at a furin recognition site (furin-like), and converted to mature-length proteins.

structure and ensures that the hydrolytic processing of proteins can occur. Members of the metzincin superfamily have significant conservation within their catalytic sites, but characteristic structural differences of individual proteins may determine their specificity for substrates and/or proteinase inhibitors.[28] Alterations in the active site consensus sequence of a number of ADAM proteins (e.g., ADAMs 2, 3, 22 and 23)[16] renders them proteolytically inactive.[20] The disintegrin-like domain of ADAM proteins (60 to 90 amino acids) has sequence similarity to the snake venom metalloproteinases (SVMPs); however, unlike SVMPs, ADAM proteins are not true disintegrin proteins because they usually lack an RGD consensus sequence.[27] This motif allows disintegrins to interact with integrins from different cell systems,[29] including platelet integrins.[18] ADAM15 is the only ADAM protein known to have an RGD motif,[29] the remainder contain an xCD motif in their disintegrin-like domains, which has also been identified as an integrin-binding motif.[30] Additionally, a number of ADAM proteins (e.g., ADAMs 1, 2, 3, 9 and 12) contain an Rx_6DEVF sequence in this domain, which binds readily to α9β1 integrins. ADAM15 and 17 lack this aspartic acid-containing sequence and consequently cannot bind α9β1 integrins.[31]

Little is known about the function of the cysteine-rich domain of ADAM proteins (~160 amino acids), but in ADAM12, for example, it interacts with cell-surface heparan sulphate proteoglycans (HSPGs), such as syndecan, to mediate cell-cell or cell-matrix attachment.[32] Interestingly, the disintegrin-like domain of ADAM12 is not involved in cell adhesion,[33] suggesting that the cysteine-rich domain compensates for its dysfunctional disintegrin-like domain. The cysteine-rich domains of ADAM1 and ADAM12 contain a putative fusogenic peptide, suggesting a role in cell-cell fusion;[34] however, currently this function remains hypothetical.

The 40 amino acid epidermal growth factor (EGF)-like domain of ADAM proteins contains six cysteine residues, and may allow ADAM proteins to interact with chaperones involved in biosynthesis.[29] Little else is known about the functions of this domain.

The majority of ADAM proteins are type I membrane proteins, and as such are anchored to the cell surface via a transmembrane (TM) domain located near the C-terminus of the protein. Proteins present in this location are mature-length proteins, many of which are catalytically active. However, some ADAM proteins (e.g., ADAMs 11, 12, 17 and 28) have alternative splice forms, which are altered upstream of the TM domain and consequently are present as soluble secreted forms.[29]

The cytoplasmic tail domain of ADAM proteins is highly variable in both length (40–250 amino acids) and sequence, and contains specialised motifs with hypothesised involvement in the signal transduction between the interior and exterior of the cell, and vice versa. The most frequently occurring motif in this domain, PxxP, acts as a binding site for SH3 (Src homology 3) domain-containing proteins (e.g., signalling adapters and enzymes) and allows protein-protein interactions at a site other than a catalytic site.[18,29] Many human ADAMs contain this motif, including ADAMs 7, 8, 9, 10, 12, 15, 17, 19, 22, 29 and 33.[18,29]

Some ADAM proteins (e.g., ADAM12 and 15) also contain potential serine-threonine and/or tyrosine kinase phosphorylation sites in their cytoplasmic tails.[18] These sites may function as ligands for SH2 domain-containing binding proteins, in addition to providing an adaptor function allowing the assembly of protein complexes required for ADAMs to execute their functional activity.[29]

ADAMs and cancer

ADAMs and cancer cell proliferation

Physiological cell proliferation is tightly regulated and responsive to the specific needs of the human body. However, when these controls become defective in a cell, it can grow and divide in an unregulated manner forming a mass of cells with no physiological function – a tumour.[35] The speed at which primary and metastatic tumours develop depends largely on the rate of cell proliferation within the tumour, and the rate of cell death.[36]

Within a tumour mass there are potentially four kinetic types of neoplastic cell, the growth fraction of proliferating cells, quiescent (or G0) cells, differentiated cells and necrotic cells. Of these fractions, the proliferating cells are the major contributor to the tumour volume, but the quiescent cells pose a significant threat of recurrent disease due to their clonogenic potential to repopulate a tumour in regression.[36]

The over-expression of certain proteolytically active ADAM proteins has been associated with the increased proliferative capacity of a number of tumour types, via the cleavage of growth factors or cell surface proteins, examples of which include ADAM9,[37] 10, 12 and 17.[20]

Certain ADAM enzymes, including ADAM9, 10,[38] 17[39] and 19[40] have α-secretase activity and shed a non-amyloidogenic fragment of amyloid precursor protein (sAPP) from the cell surface. Recombinant or purified sAPP has been shown to have a proliferation-promoting effect on skin keratinocyte,[41] rat thyroid epithelial[42] and colon carcinoma[43] cell lines. Hence, the over-expression of ADAMs with α-secretase activity by tumours, in conjunction with APP, could result in the increased proliferation of tumour cells. Elevated expression of APP messenger RNA (mRNA) and protein in oral squamous cell carcinoma, as well as the growth inhibitory effect of an anti-sense oligonucleotide against APP on a squamous carcinoma cell line, have been reported.[44]

Enhanced secretion of sAPP in explant cultures of anaplastic astrocytomas and glioblastomas has also been shown and this correlated with the malignancy of tumours.[45] ADAM10 expression is elevated in oral squamous cell carcinoma tissues and cell lines.[44] An ADAM10 anti-sense oligonucleotide reduced both the expression of this enzyme and the growth of an oral squamous cell carcinoma cell line OECM1 without changes in the secreted form of APP.[46] This might suggest mechanisms that do not involve sAPP in the cell growth-promoting activity of ADAM10.

A role for ADAM10 in the increased proliferation of tumours was, however, established by Shtutman *et al*,[47] who found that ADAM10 modulated β-catenin signalling through E-cadherin shedding. ADAM10-mediated E-cadherin shedding results in the nuclear translocation of β-catenin where it binds to transcription factors of the lymphocyte enhancer-binding factor 1/T-cell factor pathway, resulting in the increased expression of genes involved in the control of cellular proliferation, such as cyclin D1 and

c-myc.[48] This may also result in the promotion of neoplastic conversion, where progression into the cell cycle is unregulated.[47]

Another mechanism that links ADAM10 to an increase in the transcriptional activity of β-catenin involves receptor protein tyrosine phosphatases. The phosphatase intracellular portion (PIC), generated in a process that requires ADAM10, binds to and dephosphorylates β-catenin.[49] This stabilises β-catenin and results in an increase in its activity.

ADAM9 is widely expressed in humans, with over-expression in several cancers, including pancreatic cancer,[50] stomach cancer,[51] skin melanoma[52] and hepatocellular carcinoma.[53] It has been implicated in the ectodomain cleavage of heparin-binding epidermal growth factor (HB-EGF), a potent inducer of tumour growth and angiogenesis.[54]

Peduto *et al*.[55] provided compelling evidence that ADAM9 contributes to epithelial cell proliferation in mouse models of prostate carcinoma, where over-expression of ADAM9 was shown to enhance the cleavage and release of EGF and fibroblast growth factor receptor 2iiib (FGFR2iiib) from cells. The combined effect of this was increased epidermal growth factor receptor (EGFR) activation, which is linked to the proliferation of epithelial and stromal prostate cells, and inactivation of FGFR2iiib due to the shed soluble ectodomain of this receptor functioning in a dominant negative manner to disrupt FGF signalling *in vivo*.[55]

ADAM12 is associated with at least nine human cancers, including bladder,[56] breast, colon[33] and lung,[57] where it is expressed by tumour cells, and in liver carcinoma[58] where its over-expression is in stromal cells.[59] However, in stomach cancer[51] and glioblastoma (cancer originating from glial cells of the brain)[60] ADAM12 produced by tumour cells has an identified role in cell proliferation.[59] This is linked in glioblastoma cells to HB-EGF shedding by ADAM12-L, the catalytically active, long membrane-spanning variant of ADAM12.[54] Inhibition of ADAM12-L by an ADAM inhibitor (KB-R7785) inhibited proHB-EGF processing in glioblastoma tissue, and decreased the proliferation of glioblastoma cells.[60]

ADAM17 also influences tumour cell proliferation when over-expressed in cancers such as breast,[61] ovary,[62] kidney,[63] colon,[64] prostate[65] and primary hepatocellular carcinomas.[66] A mechanism proposed by Itabashi *et al*.[66] suggested that this increased cellular proliferation is mediated by ADAM proteins, particularly ADAM17, via EGFR signal transactivation triggered by angiotensin II (Ang II) stimulation. The direct stimulation of EGFR by ligand binding results in the dimerisation and subsequent phosphorylation of the two receptor molecules. This creates phosphotyrosine docking sites to activate intracellular signalling cascades, such as mitogen-activated protein kinases (MAPKs), the phophoinositide 3-kinase/Akt pathway and modulation of ion channels.[67] Ang II acts as a potent growth factor of vascular smooth muscle cells and certain cancer cell lines in addition to its fundamental role as a vasoconstrictor controlling cardiovascular function and renal homeostasis.[66] Similarly, amphiregulin (a ligand of EGFR) is released by ADAM17[68] and enhances proliferation of cancer cells.[20]

ADAMs and cancer-associated angiogenesis

The process of angiogenesis, whereby new blood vessels are formed from pre-existing vasculature, appears to provide the primary form of vascularisation within a tumour and is the rate-limiting step in cancer progression.[69] Angiogenesis has two clear functions in cancer progression, the first and most apparent role in this pathology being to provide the

Table 1. Aberrant ADAM expression in human cancers and their functions.

	Proteolytic activity	Integrin-binding	Functions in cancer	Expression in cancer*
ADAM8	Yes		Promotes cell migration and invasion	Brain,[123] Kidney,[63] Lung,[123] Pancreas,[125] Prostate[126]
ADAM9	Yes	α2β1, α6β1, α6β4, α9β1, αVβ5[37,38]	Promotes cell proliferation, adhesion and invasion	Breast,[127] Kidney,[128] Liver,[53] Lung,[129] Pancreas,[50] Skin,[52] Stomach[51]
ADAM10	Yes		Promotes cell growth, proliferation and migration	Colon,[130] Oral cavity,[46] Ovary,[131] Prostate,[132] Stomach,[133] Uterus[131]
ADAM12	Yes	α4β1, α9β1[37]	Promotes cell growth, proliferation and invasion. α4β1 binding inhibits cell migration	Bladder,[56] Bone,[134] Brain,[60] Breast,[33] Colon,[33] Liver,[58] Lung,[57] Stomach[51]
ADAM15	Yes	α5β1, αVβ3[37] α9β1[31]	Promotes cell growth and angiogenesis. αVβ3 binding inhibits cell migration	Breast,[135] Lung,[136] Ovary,[14] Prostate,[135] Stomach[51]
ADAM17	Yes	α5β1[37,38]	Promotes cell growth, proliferation and angiogenesis. α5β1 binding inhibits cell migration	Brain,[137] Breast,[61] Colon,[64] Kidney,[63] Liver,[66] Ovary,[62] Pancreas,[71] Prostate[65]
ADAM19	Yes	α4β1, α5β1, α9β1[37]	Promotes cell proliferation. α5β1 or α9β1 binding inhibits cell migration	Brain,[123] Kidney[63]
ADAM23	No	αVβ3[37,86]	Promotes cell growth and migration	Brain[86]
			Promotes cell migration	↓Breast[138]
ADAM28	Yes	α4β1, α4β7, α9β1[37]	Promotes cell proliferation	Breast,[139] Kidney,[63] Lung[140]

* Up-regulated expression of ADAM proteins in cancer, unless otherwise stated.

↓ Down-regulated expression.

tumour with its own blood supply.[70] The new vascular network supplies nutrients and oxygen throughout the tumour mass, enabling it to grow beyond the critical 2-mm sphere of an avascular tumour.[35] The second, more subtle role for neoplastic angiogenic vasculature is to provide a route for dissemination of tumour cells to different sites of the body via the process of metastasis.[69]

An increasing number of ADAM proteins have been linked to angiogenesis, at least indirectly, with potential roles in the modulation of angiogenic factors and the release of membrane-bound angiogenic inhibitors.[69]

ADAM17 is over-expressed in a number of human cancers, including pancreatic ductal adenocarcinoma,[71] breast[61] and colon carcinomas,[64] where a role as a positive regulator in tumour-associated angiogenesis has been established.[72] The combined approach of immunohistological and mRNA analysis applied by Blanchot-Jossic *et al*.[72] showed that ADAM17 is over-expressed in both its pro- and active forms in neoplastic and endothelial cells (EC) within primary colon carcinomas relative to paired normal colonic mucosa. They also demonstrated that phosphorylated EGFR (*P*-EGFR) was significantly up-regulated in most colon carcinomas compared with paired normal mucosa.

Although the relatively weak over-expression of *P*-EGFR did not correlate with ADAM17 over-expression, EGFR protein was co-expressed with ADAM17 in cancer cells and EC present in the tumour mass. This indicated that ADAM17-mediated EGFR activation is involved in tumour-mediated angiogenesis, as the downstream signalling cascade of EGFR is involved in a number of essential angiogenic processes such as cell migration, adhesion and proliferation. However, the ADAM family of proteins shows redundancy in substrate specificity, and ADAMs 9, 10, 12 and 15 have also been shown to shed EGFR ligands from the cell surface in response to stimulants;[68] hence, any of these may also be capable of EGFR-mediated angiogenesis.[13]

Further evidence of ADAM protein involvement in the positive regulation of angiogenesis was gleaned using *in vitro* models of angiogenesis. The human mammary epithelial cell line HMEC-1 expresses both ADAM17 and ADAM15, and treatment with the ADAM-specific inhibitor GL129471 inhibited the major processes involved in angiogenesis, namely migration, adhesion and proliferation, and the formation of capillary tubules.[73] The same angiogenic responses could also be inhibited in ECs by blocking the interaction between the disintegrin-like domain of ADAM15 and the angiogenic integrin α5β1 in humans.[74]

ADAM10 and ADAM17 are involved in the ligand-dependent activation of the Notch signalling pathway. This is a two-step process of controlled proteolysis in which the first cleavage is performed by ADAM10 or ADAM17.[75] The involvement of Notch in cancer depends on the cellular context and it has been proposed that it can act either in a tumour-promoting or a tumour-suppressive fashion. Oncogenic signals of Notch have, for example, been reported in breast epithelium, melanocytes and T-cell acute lymphoblastic leukaemia.[76] Through its effects on gene expression, cancer processes modulated by Notch include suppression of p53, angiogenesis and cell adhesion.[77] However, it has also been reported that conditional Notch1 knockout mice develop cutaneous lesions that resemble basal cell carcinoma.[77]

ADAMs and cancer cell adhesion and migration

Cell migration is a complex sequential process necessary for physiological development, tissue repair and regeneration.[37] It is also the process that drives the metastasis of cancer cells. Cell migration is aided by the integrin family of adhesion molecules, which promote stable interactions between cells and the ECM, as well as functioning as signalling molecules initiating intracellular signals that regulate certain cell behaviours including cell migration.[37]

A number of ADAM proteins interact with cell surface integrins via their disintegrin-like domain, and it is possible that these interactions influence cell migration during cancer progression. Activated hepatic stellate cells, commonly known as liver stromal cells, secrete the soluble splice variant of ADAM9 (ADAM9-S), which can localise to the surface of colon carcinoma cells via an interaction between α6β4 and α2β1 integrins on the tumour cell and the distintegrin domain of ADAM9-S. Its localisation to the cell surface can promote the invasion of colon carcinoma cells *in vitro* by the degradation of laminin and other ECM components,[78] but further investigation is required to determine whether or not this effect is also observed *in vivo*. This highly invasive phenotype has also been demonstrated in a variety of cell lines.[37]

Interestingly, recent crystallographic studies have revealed that the disintegrin domain of ADAMs, which supposedly interacts with integrins, is inaccessible for protein binding. The hypervariable region of the cysteine-rich domain has been proposed as a potential protein-protein association region.[79]

ADAM10 can critically affect the adhesive properties of epithelial cancer cells by the proteolytic processing of E-cadherin both *in vitro* and *in vivo*.[48] The resultant soluble E-cadherin disrupts cell-cell adhesions and induces cell invasion into collagen type I.[48,80] Transfection of epithelial cells (HaCaT cells) with ADAM10 resulted in increased migration due to the abrogation of cell-cell contacts, whereas inhibition of ADAM10 resulted in increased adhesiveness of these cells and consequent reduction of cell migration.[48] Similarly, an 80 kDa fragment of E-cadherin present in the serum of prostate cancer patients with metastatic disease is associated with the increased levels of several adamalysin proteins, including ADAM12 and ADAM15.[81] This may demonstrate the substrate redundancy of this family of proteins, with a number of ADAM proteins being capable of processing E-cadherin.

ADAM10 is also involved in the ectodomain cleavage of the adhesion molecule L1 in ovarian and uterine carcinomas where both ADAM10 and L1 are over-expressed. The resultant soluble L1 stimulates the migration of neural and tumour cells through autocrine/paracrine binding to αVβ5 integrin.[82] Gutwein *et al*.[83] showed that L1 shedding can occur either at the cell surface or in secretory vesicles derived from the Golgi apparatus, with its release enhanced when ADAM10 is over-expressed and completely blocked when ADAM10 is inactivated by recombinant dominant-negative ADAM10. This is the first time ADAM10 has been implicated as a vesicle-based proteinase and the implications of this are important; tumour-derived vesicles, which contain an assortment of proteolytic enzymes and other proteins including L1, help tumour cells to infiltrate, metastasise and sculpt their microenvironment.[83] ADAM10 also appears to be the major L1 sheddase in lymphoma, lung

carcinoma and melanoma, where it also increases cell migration and invasion.[54]

Recently, it has been shown that ADAM15 cleaves E-cadherin in response to growth factor deprivation. A soluble E-cadherin fragment bound to ErbB in breast cancer cells stimulated cell migration and proliferation through the Erk signalling pathway.[84] Inhibition of expression of ADAM15 in a prostate cancer cell line (PC-3) decreased cell migration and adhesion to defined extracellular matrix proteins.[85] A concomitant reduction in the cleavage of N-cadherin by ADAM15 at the cell surface was observed.

ADAM23 has a proteolytically inactive metalloproteinase domain, but can promote cell migration by functioning as an adhesion molecule. ADAM23 is specifically involved in αVβ3-mediated cell-cell interactions that occur in physiological and pathological processes to promote the migration of neural derived cells, including that of neuroblastomas and astrocytomas.[86]

Conversely, the interaction of ADAM proteins with integrins can inhibit cell migration; for example, the interactions of ADAM12 with α4β1, ADAM15 with αVβ3, ADAM17 with α5β1 and ADAM19 and ADAM33 with both α5β1 and α9β1 all resulted in inhibitory effects on CHO cell migration.[37,87] The mechanisms by which these inhibitory effects are mediated may vary, but are as yet poorly understood. However, ADAM15 over-expression in ovarian cancer disturbed the pro-migratory interaction of αVβ3 integrin with vitronectin, which resulted in reduced cellular adhesion to vitronectin and the consequent reduction in random cellular motility.[14]

A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) proteins

ADAMTSs are multi-domain, extracellular proteins belonging to the same subfamily of metzincin proteins as the ADAMs, the adamalysins.[11] Although they are secreted proteinases, they usually bind to ECM components such as HSPGs.[88] There are 19 ADAMTSs, numbered 1–10 and 12–20 (ADAMTS-11 is the same protein as ADAMTS-5) with known functions in ECM processing, organogenesis, haemostasis[88] and angiogenesis.[89] They can be divided into four subdivisions depending upon their structural characteristics and activities.[90]

The first division contains ADAMTS-1, -4, -5, -8, -9, -15 and -20, which possess the ability to cleave members of the hyalectan or lectican family of large aggregating proteoglycans, including aggrecan, versican, neurocan and brevican,[91] and are therefore known as hyalectanases. All ADAMTSs in this division can cleave aggrecan, with the exception of ADAMTS-20, which originally led them to be known as aggrecanases.

The second group containing ADAMTS-2, -3 and -14 are known as pro-collagen N-propeptidases; ADAMTS-2 is able to cleave type I, type II and type III procollagens, ADAMTS-3 processes type II procollagen peptides, and ADAMTS-14 (a homologue of ADAMTS-2) functions as the major type I procollagen N-propeptidase in tendons.

The third group contains only ADAMTS-13; this proteinase is responsible for the cleavage of the large multimeric von Willebrand factor (VWF) precursor. The remaining ADAMTS proteins are grouped into a category known as 'others', which can be subgrouped into four pairs based on their structural features; these are ADAMTS-6 and -10, ADAMTS-7 and -12, ADAMTS-16 and -18, and ADAMTS-17 and -19.[90]

ADAMTS domain structure

ADAMTSs share considerable structural similarities with ADAMs, with four domains of similar type, a prodomain, a metalloproteinase domain, a disintegrin-like domain and a cysteine-rich domain (Fig. 1). Characteristic domains of ADAMTSs are thrombospondin type-I repeats (TSRs),[92] a spacer domain and several C-terminal domains unique to particular ADAMTSs.

Like ADAMs, ADAMTSs are synthesised as zymogens; however, after proteolytic processing at the N-terminus to remove the signal sequence and prodomain, they are secreted from cells. The prodomain has similar functions to the prodomain of ADAMs, but is also involved in secretion from cells. While for most ADAMTSs the removal of the prodomain is an important step in their activation, ADAMTS-13 is enzymatically active when this region is still attached.[93] As for ADAMs, the main enzyme involved in the removal of the prodomain is furin. However, the prodomain of ADAMTS-4 can be removed in a cell line that does not express furin;[94] therefore, other enzymes may also be involved.

ADAMTSs possess a metalloproteinase domain with a zinc binding module of the sequence HExxHxxGxxHD similar to the ADAM proteins. The disintegrin-like domain of ADAMTSs is 25–45% similar to that of SVMPs; however, none of the ADAMTSs contain an RGD motif and their interactions with integrins have not been reported. The central TSR that follows is very similar in all ADAMTSs. Linked to it is a cysteine-rich domain that contains 10 conserved cysteines and is also highly homologous between ADAMTSs. The spacer domain is the least homologous of all the domains and it comprises an N-terminal part in which several hydrophobic amino acids are conserved and a C-terminal section which is highly variable.

The TSRs closer to the C-terminus of ADAMTSs differ much more in amino acid sequence than the central TSR. They can include a CVSTCG motif that binds to the CD36 cell surface receptor or a motif known to interact with sulphatide and heparin. Members of the ADAMTS family have a different number of C-terminal TSRs (e.g., ADAMTS-4 lacks a C-terminal TSR motif, while ADAMTS-9 and ADAMTS-20 have 14 C-terminal TSRs).[95] In several ADAMTSs the TSRs and spacer domain have been shown to be involved in binding to the ECM.

Some ADAMTSs have unique C-terminal domains. ADAMTS-2, -3, -10, -12, -14, -17 and -19 have a protease and lacunin (PLAC) domain.[96] ADAMTS-13 is characterised by the presence of two complement subcomponent C1r/C1s/embryonic sea urchin protein Uegf (CUB) domains which also occur in proteases of the astacin family (a subfamily of the metzincins).[97] The CUB domain is present in several extracellular and plasma membrane- bound proteins. The long isoform of ADAMTS-9 and ADAMTS-20 have a gon domain that was discovered originally in an ADAMTS involved in the development of gonads in *Caenorhabditis elegans*.[98] ADAMTS-7 and ADAMTS-12 contain a mucin domain which is located between their C-terminal TSRs (specifically between repeats 3 and 4 out of seven).[99]

Table 2. Aberrant ADAMTS protein expression in human cancers and their functions.

	Proteolytic activity	Functions in cancer	Expression in cancer*
ADAMTS-1	Yes	Promotes cell invasion and angiogenesis	Breast[116]
		Permits angiogenesis	↓Breast,[88] ↓Liver,[103] ↓Lung,[57] ↓Pancreas[103]
ADAMTS-4	Yes	Promotes cell invasion	Brain[113]
ADAMTS-5 or ADAMTS-11	Yes	Promotes cell invasion	Brain[113]
ADAMTS-8	Yes	Undetermined	Lung[141]
		Permits angiogenesis	↓Brain[106]
ADAMTS-13	Yes	Undetermined	↓Brain[117]
ADAMTS-15	Yes	Predictor of prolonged survival	Breast[116]
		Predictor of poor prognosis	↓Breast[116]
ADAMTS-18 or ADAMTS-21	Yes	Tumour suppressor	Oesophagus[142]

* Up-regulated expression of ADAMTS proteins in cancer, unless otherwise stated.

↓ Down-regulated expression.

ADAMTSs may also undergo C-terminal processing post-translationally, which can alter their localisation and substrate specificity. C-terminal processing has been shown for ADAMTS-1, ADAMTS-4, ADAMTS-8, ADAMTS-9 and ADAMTS-12. Controlled proteolysis usually takes place in the spacer domain; however, in ADAMTS-12 it is in the mucin domain. ADAMTS-4 is well characterised in terms of a relationship between C-terminal processing, localisation and biological activity. C-terminal processing of ADAMTS-4 converts the full-length 75-kDa form to 60-kDa and 50-kDa species. This is accompanied by changes in binding to the ECM, the pattern of cleavage of aggrecan and the range of substrates degraded.[100] While the 75-kDa isoform associates with the ECM, shorter forms with truncated spacer regions do not. It has also been shown that the TSR, which precedes the spacer and cysteine-rich domain, is important for ADAMTS-4 binding to sulphated GAGs linked to aggrecan.

ADAMTSs and cancer

ADAMTSs and cancer-associated angiogenesis

Until recently all proteinases were considered to be positive regulators of tumoural angiogenesis. However, four members of the ADAMTS family have recently been shown to have anti-angiogenic properties. ADAMTS-1 (METH-1) and ADAMTS-8 (METH-2) exhibit potent angio-inhibitory activity *in vitro*.[13] Both enzymes act independently to inhibit bovine vascular endothelial growth factor (bVEGF)-induced vascularisation in the rabbit corneal pocket assay and inhibit VEGF-induced angiogenesis in the chick chorioallantoic membrane (CAM) assay.[64]

The anti-angiogenic activity of ADAMTS-1 has been mapped to the three TSRs in the protein's C-terminus. Recombinant and proteolytic fragments containing these repeats also exhibit angio-inhibitory activity in rabbit corneal pocket and chick CAM assays.[64] However, mutational analyses have revealed that although TSRs are necessary for the inhibition of angiogenesis, alone they are not sufficient to bring about this response *in vivo*. The spacer domain must be present in combination with the TSRs of the protein to elicit an anti-tumour response.[101] Furthermore, a GWQRRL/TVECRD motif common to the first C-terminal TSR of both ADAMTS-1 and -8, but absent from all other ADAMTS proteins, may play an important role in the angio-inhibitory action of these proteins.[89] C-terminal processing of ADAMTS-1 from its 87-kDa full-length form to a 65-kDa form lacks the terminal TSR domain, and part of the spacer domain reduces its angio-inhibitory effect.[102]

The sequestration of VEGF165 by ADAMTS-1 and -8 may provide a mechanism by which they execute their anti-angiogenic activity.[69] $VEGF_{165}$ is one of the most specific mediators of tumour angiogenesis, with suppression of VEGF signalling causing the inhibition of angiogenesis and an associated reduction in tumour burden. Conversely, the over-expression of VEGF and its receptor, VEGFR2, results in the increased invasion and metastasis of human cancers.[103]

Luque *et al*.[103] have shown that ADAMTS-1 can bind to $VEGF_{165}$ and form a stable complex, but it cannot bind to the splice variant of VEGF lacking a heparin-binding domain in its C-terminal ($VEGF_{121}$). Interestingly, the TSRs of ADAMTS-1 and -8 contain the consensus sequence WSxWS, which also binds heparin.[64] So it is likely that heparin or another HSPG, such as syndecan, acts as a chaperone between ADAMTS-1 and $VEGF_{165}$, resulting in the reduced bioavailability of VEGF and the consequent inhibition of VEGFR2 phosphorylation. This leads to decreased endothelial cell proliferation and angiogenesis.[103] However, the functional inactivation of VEGFR2 due to the binding of ADAMTS-1 to $VEGF_{165}$ is reversible, and dissociation of the complex results in an active growth factor and the subsequent phosphorylation of VEGFR2.[103,104]

In order to overcome the anti-angiogenic actions of ADAMTS-1 and -8, many tumour types have been found to down-regulate their expression. For example, ADAMTS-1 is down-regulated in mammary,[88] hepatocellular and pancreatic carcinomas,[105] and ADAMTS-8 in brain tumours.[106]

In contrast, the over-expression of full-length ADAMTS-1 in TA3 mammary carcinoma, Lewis lung carcinoma[107] and Chinese hamster ovary (CHO) cell lines[101] was found to promote angiogenesis and invasion. This must suggest that C-terminal processing, and consequently the proteolytic status of ADAMTS-1, determines its effect on tumour metastasis *in vivo*.[20,107]

Another potential anti-angiogenic ADAMTS protein is ADAMTS-5, and although the function of full-length

ADAMTS-5 in angiogenesis is presently unknown, the first TSR of ADAMTS-5 functions as an angiogenesis inhibitor *in vitro*.[108] Synthetic and recombinant forms of the centrally located ADAMTS-5 TSR, but not the C-terminal TSR, inhibited EC tubule formation on Matrigel, a consequence of reduced cell-matrix attachment and increased EC apoptosis. The first TSR peptide also inhibited EC proliferation in the presence and absence of VEGF, which normally stimulates EC proliferation, although this did not contribute significantly to the decrease in EC tube-like structures.[108] However, unlike other known anti-angiogenic proteins, the first TSR peptide of ADAMTS-5 promotes the migration of ECs, and it is hypothesised that this increased motility may decrease the ability of ECs to form organised tubules.[108]

Llamazares *et al*.[109] provided extensive evidence that ADAMTS-12 functions as an angio-inhibitory protein. ADAMTS-12-expressing clones of Madin-Darby canine kidney (MDCK) cells were resistant to the effects of hepatocyte growth factor (HGF), which normally induces MDCK cell proliferation and migration. The ADAMTS-12-expressing MDCK clones did not undergo cell scattering but maintained cell-cell contacts and formed epithelial-like colonies. Evaluation of key components of the HGF signalling pathway by Western blot analysis determined that levels of active phosphorylated extracellular signal-regulated kinase (*P*-ERK) were significantly reduced in ADAMTS-12-expressing MDCK cells, compared with control MDCK cells. Also, E-cadherin was detectable in ADAMTS-12-expressing MDCK cells but not in control cells following HGF stimulation, and vimentin was absent from ADAMTS-12-expressing MDCK cells but present in control cells after HGF stimulation. These results are indicative of ADAMTS-12 expression negatively regulating the HGF signalling pathway. This is further supported by the findings that HGF mediates the formation of epithelial tubules in MDCK cells, but ADAMTS-12-expressing MDCK cells fail to undergo the epithelial-mesenchymal transition characteristic in tubule formation.[109]

Similarly, recombinant human ADAMTS-12 abolished the ability of bovine aortic endothelial (BAE-1) cells to form VEGF-induced capillary structures *in vitro*. Furthermore, the use of the human lung adenocarcinoma cell line A549, which is capable of forming primary tumours in immunodeficient mice, has led to the proposition that ADAMTS-12 may confer antitumour properties *in vivo*. It was observed that tumours originating from a stable ADAMTS-12-expressing A549 clone, A549-TS12, in severe combined immunodeficiency (SCID) mice had a significantly reduced growth rate in comparison to A549-derived tumours.[109]

ADAMTSs and cancer progression

A number of ADAMTS proteins have been implicated in the progression of cancer, but a specific role in this progression has yet to be elucidated. These include ADAMTS-4, -5, -8, -13 and -15.

Human glioblastomas are the most common type of brain tumour and also the most difficult to treat effectively due to their infiltrative invasion of surrounding normal neural tissue.[110] The ECM can modulate cellular movement, as is the case for glioblastomas,[111] which consistently up-regulates the ECM protein brevican, a neural-specific chondroitin sulphate proteoglycan (CSPG), also known as brain-enriched hyaluronan binding protein (BEHAB). In normal brain tissue, BEHAB/brevican inhibits cell and neurite motility, but its over-expression in glioblastomas dramatically enhances tumour growth and invasion *in vitro* and *in vivo*.[110]

An up-regulation of BEHAB/brevican cleavage products has also been observed in human glioblastomas,[112] with the N-terminal fragment containing a hyaluronan-binding domain causing increased invasive behaviour of tumours *in vivo*.[113] Hu *et al*.[114] have since shown that cleaved brevican promotes EGFR activation, increases the expression of adhesion molecules, and promotes the secretion of fibronectin and the accumulation of fibronectin microfibrils on the cell surface. Furthermore, the N-terminal cleavage fragment of brevican binds to fibronectin to promote glioblastoma cell motility in cultured cells and surgical glioblastoma samples.[114]

BEHAB/brevican is cleaved at a single site ($E_{395} – S_{396}$) by the hyalectanases ADAMTS-4 and -5, and although both proteinases are present in normal brain tissue, their production is increased in proliferating glioblastoma cells *in situ*, compared to cultured human glioblastoma cells.[115] These data have led to the conclusion that ADAMTS-4 and -5 may contribute to the highly invasive behaviour of malignant glioblastomas via the processing of BEHAB/brevican.

Eleven ADAMTS genes are reportedly dysregulated in human breast cancer,[88] two of which (high ADAMTS-8 and low ADAMTS-15) are associated independently with a poorer prognosis in this disease type.[116] ADAMTS-8 RNA expression was down-regulated in breast carcinomas of all grades and types, as compared to non-neoplastic mammary tissue.[88] However, when ADAMTS-8 RNA was expressed at higher levels, patients had a significantly poorer prognosis with a decreased overall survival time.[116] Additionally, ADAMTS-15 RNA expression may act as a predictor of prolonged relapse-free survival, as its expression was associated with smaller tumours and was significantly down-regulated in grade 3 breast carcinomas compared to grades 1 and 2.[116]

Conflicting evidence has been reported regarding the role of ADAMTS-13, previously known as von Willebrand factor (VWF)-cleaving protease, in malignant tumours. Oleksowicz *et al*.[117] and Koo *et al*.[118] observed a significant deficiency of the VWF-cleaving protein ADAMTS-13 in plasma samples from patients with advanced-stage malignant tumours (≤15%) and disseminated tumours (≤30%), respectively. Mannucci *et al*.[119] also reported differences in the ADAMTS-13 plasma levels in adult and paediatric patients with metastatic tumours compared to patients with localised tumours. However, their results showed considerable variability (18–130%).

Furthermore, Mannucci *et al*.[119] found significantly lower ADAMTS-13 levels in adults with localised tumours as compared to healthy individuals, which is in contrast to Oleksowicz *et al*.[117] where ADAMTS-13 levels were normal (≥88%). Further data presented by Böhm *et al*.[120] support those of Mannucci *et al*.[119] in that mild ADAMTS-13 deficiency was seen in tumour patients, but the deficiency was not restricted to malignant or metastatic tumours. Despite the variable data presented, all investigators[117–120] agree that some cancer patients have ADAMTS-13 deficiency, but as yet no causal association has been made between ADAMTS-13 deficiency and malignancy and/or metastasis.[120]

In summary

The dysregulated (usually elevated) expression/activity of many ADAM and ADAMTS proteins has been demonstrated in a wide range of human tumour types, and is often associated with a more aggressive tumour phenotype. No mutational defects in these enzymes had been associated with any tumour type[37] until recently, when three somatic mutations of ADAM12 were observed with significant frequency in human breast cancers.[121]

Through the actions of these enzymes, membrane-bound growth factors, transcription factors, cytokines and cell adhesion molecules can be released/activated, and ECM components degraded. All of which can contribute directly to tumour formation and dissemination by regulating tumour cell proliferation, migration, adhesion and angiogenesis. In addition, several of these enzymes also modulate, through specific processing actions, the activity of chemokines that can mediate inflammatory and immune responses during tumourigenesis..[122]

ADAMs and ADAMTSs are potential targets in anticancer therapy and inhibitors directed against them are being developed and tested. However, a more thorough understanding of the exact role of each enzyme in cancer is necessary in order to focus on more specific targets and avoid problems associated with broad-spectrum inhibitors. □

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