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Differential roles of protease isoforms in the tumor microenvironment

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Abstract
Alternative splicing of precursor mRNA is a key mediator of gene expression regulation leading to greater diversity of the proteome in complex organisms. Systematic sequencing of the human genome and transcriptome has led to our understanding of how alternative splicing of critical genes leads to multiple pathological conditions such as cancer. For many years, proteases were known only for their roles as proteolytic enzymes, acting to regulate/process proteins associated with diverse cellular functions. However, the differential expression and altered function of various protease isoforms, such as (i) anti-apoptotic activities, (ii) mediating intercellular adhesion, and (iii) modifying the extracellular matrix, are evidence of their specific contribution towards shaping the tumor microenvironment. Revealing the alternative splicing of protease genes and characterization of their protein products/isoforms with distinct and opposing functions creates a platform to understand how protease isoforms contribute to specific cancer hallmarks. Here, in this review, we address cancer-specific isoforms produced by the alternative splicing of proteases and their distinctive roles in the tumor microenvironment.

Keywords Protease · Alternative splicing · Cancer · Tumor microenvironment

1 Introduction

Advances in proteogenomic approaches have facilitated the identification and characterization of the complete protease repertoire of different organisms [1]. This coordinately regulated repertoire of proteases which modulates the cell/tissue local environment is known as the cell/tissue degradome [2]. At least 569 proteases are identified in the human degradome (excluding protease pseudogenes and retrovirus-derived protease-related sequences) and are classified into 68 families. Based on the mechanism of catalysis, five distinct protease classes were identified, including metalloproteases (MMPs) and serine proteases with 194 and 176 members, respectively, followed by 150 cysteine, 28 threonine, and 21 aspartic proteases [3]. Through irreversible hydrolytic reactions, proteases can regulate the activity and fate of cellular proteins by directing intra- or extracellular localization, protein shedding, activation/inactivation of cytokines, hormones and growth factors, transformation of receptor agonists to antagonists, and exposure of cryptic neoproteins [1, 4]. Thereby, proteases are uniquely responsible for controlling crucial biological processes, such as cell–cycle progression, DNA replication, immunity, and apoptosis, by modulating bioactive molecules involved with such cellular processes. Over the past three decades of cancer research, investigators have described the role of proteases in almost all the stages of cancer progression [5–7]. Proteases which are functionally involved with any stage of cancer progression are collectively known as the cancer degradome [2]. Tumor-associated proteolytic activity of proteases is mainly attributed to the degradation of extracellular matrix (ECM) components, such as laminins, fibronectin, collagens I and IV, elastin, and other structural proteins of basement membranes, which facilitates the subsequent invasion of the tumor into the surrounding normal tissue [6, 8]. Several secreted members of the serine, cysteine, and MMP sub-classes are associated with pro-metastatic activities in

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various type of cancers [6]. Protease involvement with cellular invasion was also reported, as indicated by the gain-of-function and loss-of-function experiments which used both experimental and spontaneous metastatic models [3]. In particular, proteases with tumor-suppressive and oncogenic activities are reported with altered expression levels at primary as well as metastatic sites of the tumor growth [9].

Over the past two decades, transcriptomic studies have discovered altered splice variants of key regulatory genes that contribute to the development of many diseases, including cancer [10, 11]. Different modes of alternative splicing events, such as (i) intron retention, (ii) exon skipping, (iii) alternative 5′/3′ splice sites, (iv) alternative promoter selection, and (v) alternative polyadenylation sites, create an opportunity to produce protein isoforms having distinct and opposing functions from a single gene [10, 12]. Currently, many protease isoforms evolved due to alternative splicing and have been recognized with specific roles in facilitating the growth and progression of many tumors. Some of these protease isoforms were identified with key roles in modulating responses to anti-cancer therapy [13]. In this review, we focus on key alternative splicing events of protease genes and differential roles of protease isoforms in the tumor microenvironment.

2 Alternative splicing of proteases in the tumor microenvironment

The diversity of human protease function directly results from the evolutionary intervention of variety of enzymes with diverse structures and shapes. Structural design of proteases ranges from small catalytic units (~ 20 kDa) to sophisticated proteolytic enzymes (~ 0.7–6 MDa) [3]. The main functional domain of proteases is the catalytic domain which executes the proteolytic reaction: hydrolysis of peptide bonds. Many proteases link their catalytic domain with other specialized structural and functional (non-catalytic) domains, such as (i) pre-peptide/signal peptide domain: guiding their cellular localization; (ii) auto-inhibitory pro-peptide domain: preventing premature activation; (iii) ancillary domains: facilitating homo or heterotypic interactions with specific substrates, receptors, protease inhibitors; (iv) protein interaction domains, such as disintegrin domain, death effector domains, and caspase recruitment domains; and (v) transmembrane and cytoplasmic domains: anchoring the enzyme to the lipid membrane and binding to cytoskeleton or regulatory factors, respectively [3].

The complexity of proteases is further increased through the alternative splicing and differential polyadenylation of protease genes, causing changes in their pre-pro-enzyme amino acid (aa) sequence. Consequently, such changes directly alter their (i) cellular localization, (ii) interaction with substrates and other proteins, (iii) proteolytic activity, and (iv) sensitivity to endogenous inhibitors. Also, differences in the length of alternative splice variants can affect their stability, as well as their translation efficiency [14–16]. Hence, alternative splicing creates an interesting platform to produce protease isoforms with altered activities, causing numerous disease conditions, including cancer. It has long been known that tumor growth and progression are often facilitated by the flexibility of protease genes to produce isoforms with opposing functions [12, 17]. Earlier findings raised the possibility that part of the heterogeneity of human tumors may have been caused by the opposing and distinct roles of protease isoforms, resulting from the alternative splicing machinery [16].

So far, many protease splice variants and their isoforms have been characterized based on their structure and their proteolytic potential and found differentially expressed in many cancer cell lines and tissues (Table 1). Even though some protease isoforms such as that of Kallikreins described in this review are yet to be functionally characterized, their potential importance towards cancer diagnostics and therapeutics should not be ignored. Below we describe the splicing events of some cancer-specific proteases in detail.

2.1 Kallikrein-related serine proteases

Serine proteases are the second largest human protease family which include Kallikrein-related (KLK) proteases and type II transmembrane serine proteases such as seprase and hepsin, and urokinases. Analogous to other serine proteases, the KLKs are comprised of signal peptide, pro-peptide, serine-protease domain/catalytic domains but not other ancillary domains [27]. The active site of the KLK catalytic domain is comprised of three amino acids: histidine, aspartate, and serine (thus, termed as serine proteases) [27]. Serine proteases are known to be responsible for coordinating various physiological functions whereas many KLK members were also reported to be associated with critical cancer hallmarks [27, 113]. Human tissue KLKs are comprised of 15 (KLK1-15) members, and many studies have presented the differential expression of KLK members in human cancers [18, 19, 27, 114–116]. Strikingly, all 15 *KLK* genes were found to undergo alternative splicing and found differentially expressed in many type of diseases including cancer [27, 115, 117]. Variations in the KLK amino acid sequence may interfere with its catalytic activity, emphasizing the structural and functional complexity of isoforms resulted from *KLK* alternative splicing [117]. In the next section, we describe four key KLK members for which isoforms have been identified and expression reported to be dysregulated in one/multiple cancers.

2.1.1 KLK3

The major catalytic isoform of KLK3 is known as prostate-specific antigen (PSA) (length, 261 aa; mass, 28.7 kDa) and found abundantly expressed by human prostate epithelial cells

Table 1 Characterization of protease splice variants and isoforms associated with the tumor microenvironment

Protease		Splice variant	Characteristics of the splice variant and isoform	Specific function of the isoform variant	Associated cancer type	Selected references
KLKs	KLK3	*PSA*	Full-length variant Codes for the catalytically active, secreted protein PSA	Degradation of ECM components—induces cancer cell invasion Generation of anti-angiogenic angiostatin-like fragments—inhibits metastasis	Prostate cancer Lung cancer, prostate cancer	[18, 19] [20, 21]
		PSA-RP1	Alternative splicing of intron 4 leads to different exons 5′ and 3′ UTR Codes for the catalytically inactive isoform PSA-RP1	Unknown function	Prostate cancer	[22, 23]
		PSA-RP2	Retention of intron 3, generating a premature stop codon Codes for the catalytically inactive isoform PSA-RP2	Increasing cell migration—induces metastasis	Prostate cancer	[22, 24]
		PSA-RP3	Alternative splicing of exon 3, which lacks 129 bp in exon 3 Codes for the isoform PSA-RP3 lacking N-glycosylation binding site for the carbohydrate chain	Unknown function	Prostate cancer	[22, 25]
		PSA-RP4	Alternative splicing of exon 3 leads to two short exons Codes for the catalytically inactive isoform PSA-RP4	Unknown function	Prostate cancer	[22]
		PSA-RP5	Alternative splicing of intron 4 leads to different exon 4 and excludes exon 5 Codes for the catalytically inactive isoform PSA-RP5	Unknown function	Prostate cancer	[22]
		PSA-SV5	Retention of intron 2 Exclusion of exons 1 and 2 Codes for truncated isoform	Unknown function	Prostate cancer	[26]
	KLK4	*KLK4*	Full-length variant Codes for the catalytically active, secreted isoform KLK4	Degradation of ECM components, pro-MMP activation, releasing growth factors—induces invasion and migration	Prostate cancer	[27]
		KLK4-205	Exclusion of exon 1 Codes for the catalytically inactive isoform KLK4-205	Unknown function	Prostate cancer Endometrial cancer	[28, 29]
		KLK4-IRIII	Contains only four exons with intron 3 retention Codes for the catalytically inactive isoform	Unknown function	Prostate cancer	[30]
	KLK6	*KLK6-A*	Full-length variant Codes for the catalytically active, secreted isoform KLK6	E-cadherin shedding, Degradation of ECM reduces cancer cell adhesion and induces invasion.	Colon cancer, breast cancer, melanoma, lung cancer	[31–33]
		KLK6-B	Differs only in the length of 5′ UTR compared with *KLK6-A* Codes for the catalytically active, secreted isoform KLK6			
		KLK6-C *KLK6-D* *KLK6-E*	Differs in the length 5′ UTR, has 3 coding exons Codes for catalytically inactive isoforms	No activation or secretion—unknown function	Breast cancer	[34]
	KLK8	*KLK8-v1*	Full-length variant Codes for the catalytically active, secreted isoform KLK8-1	Downregulating VEGF signaling—reduces cancer cell invasion	Lung cancer, ovarian cancer	[35]
		KLK8-v2	Retention of intron 2 Codes for the catalytically active, secreted isoform KLK8-2			

Table 1 (continued)

Protease		Splice variant	Characteristics of the splice variant and isoform	Specific function of the isoform variant	Associated cancer type	Selected references
		KLK8-v3 *KLK8-v4* *KLK8-v5*	Alternative splicing between exons 3 and 5 Codes for catalytically inactive isoform	No activation or secretion—unknown function	Ovarian cancers, lung cancer	[35–37]
Cathepsins	Cathepsin B	*CB (-2)*	Exclusion of exon 2 codes for the catalytic isoform CB (-2)	Intracellular and extracellular proteolysis ECM remodeling and degradation—induces cancer metastasis	Osteosarcoma, meningioma, mammary and breast cancer, glioma and glioblastoma, melanoma, cervical cancer, lung cancer	[38–48]
		CB (-2, 3)	Exclusion of exons 2 and 3 Codes for the truncated isoform tCB	Nuclear fragmentation and cell apoptosis	Melanomas, breast cancer, colon cancer	[49]
	Cathepsin L	*L-A*	Differs each other by the length of 5′ UTR (*L-A* > *L-A1* > *L-A2* > *L-A3*)	Degradation of ECM components, bone resorption, turnover of intracellular regulatory proteins—increases cancer metastasis	Kidney cancer, lung cancer, glioblastoma, breast cancer	[50, 51]
		L-A1	Codes for the full-length isoform cathepsin L		Kidney cancer, lung cancer	[51]
		L-A2			Epidermoid cancer, cervical cancer, squamous cell carcinoma, lung cancer, breast cancer, hepatoma	[50–52]
		L-A3			Kidney cancer epidermoid cancer, cervical cancer, squamous cell carcinoma, breast cancer, hepatoma	[52–54]
		L-B	Differs by the length of 5′ UTR, transcription initiation site at intron 1 Codes for the full-length isoform cathepsin L		Human adenocarcinoma, hepatoma, kidney cancer	[55]
Caspases	Caspase 2	*Caspase-2L*	Exclusion of exon 9 Codes for the truncated apoptotic isoform caspase-2L	Cellular apoptosis with tumor suppressor activity Impaired programmed cell death—induces cancer progression	Gastric and colorectal cancer, leukemia, breast cancer, ovarian cancer, glioblastoma	[56–61]
		Caspase-2s	Inclusion of exon 9 incorporated with an premature stop codon—lacks small catalytic subunit Codes for the anti-apoptotic isoform caspase-2s	Endogenous inhibitor of cellular apoptosis	Neuroblastoma, leukemia and lymphoma, ovarian cancer, cervical cancer, lung cancer	[62–65]
		CASP-2l-Pro	Alternative splicing creates a translational frameshift with a premature stop codon Codes for the isoform caspase-2l-pro	Negatively interferes with pro-caspase-2L activation and death receptor- and drug-mediated apoptoses	Lymphoma	[62]
	Caspase 3	*Caspase-3L*	Full-length variant Codes for the apoptotic isoform caspase-3L	Cellular apoptosis and ECM remodeling—increases cancer cell migration and invasiveness	Ovarian cancer, melanoma, hepatoma, breast cancer, leiomyoma, cervical cancer, neuroblastoma	[13, 66–73]
		Caspase-3s	Exclusion of exon 6—lacks part of small catalytic subunit Codes for the anti-apoptotic isoform caspase-3s	Endogenous inhibitor of cellular apoptosis	Breast cancer, neuroblastoma, cervical cancer	[13, 67, 71]
	Caspase 8	*Caspase-8a* *Caspase-8b*	Exclusion of exon 8b Codes for the apoptotic isoform caspase-8a/8b	Cellular apoptosis—induces NF-κB-mediated tumor metastasis	Neuroblastoma	[74–78]
		Caspase-8L	Inclusion of exon 8b incorporated with a premature stop codon—lacks catalytic domain Codes for the anti-apoptotic isoform caspase-8L	Endogenous inhibitor of cellular apoptosis	Undifferentiated neuroblastoma, stem cell–derived leukemia	[79, 80]
		Caspase-8h	Insertion 17 aa sequence—disrupted DED Codes for a apoptotic isoform caspase-8h	Increases cell motility	Neuroblastoma	[81, 82]
		Caspase-8s	106-bp deletion incorporated with an in-frame stop codon—lacks a part of second DED and C-terminal caspase domain Codes for the apoptotic isoform caspase-8s	Binds with FADD domains—cellular apoptosis Overexpression causes increased sensitivity to the apoptotic stimuli	Leukemia	[83]
	Caspase 9	*Caspase-9a*	Full-length variant Codes for the apoptotic isoform caspase-9a	Cellular apoptosis—tumor suppressor activity	Lung cancer, prostate cancer, bladder cancer, pancreatic cancer	[84–91]
		Caspase-9b	Exclusion of exon 3-6—lacks part of central catalytic domain	Endogenous inhibitor of cellular apoptosis	Cervical cancer, ovarian cancer, lung cancer,	[92, 93]

Table 1 (continued)

Protease		Splice variant	Characteristics of the splice variant and isoform	Specific function of the isoform variant	Associated cancer type	Selected references
			Codes for the anti-apoptotic isoform which lacks catalytic domains caspase-9b			
	Caspase 10	*Caspase-10A*	Exclusion of exons 6, 7, and 11 Codes for the apoptotic isoform caspase-10A	Cellular apoptosis—tumor suppressor activity	Neuroblastoma, colon, B lymphoma	[94]
		Caspase-10B	Inclusion of exons 6 and 7 but excludes exon 10 and contains part of exon 11 Codes for the apoptotic isoform caspase-10B		Breast cancer	[95]
		Caspase-10C	Inclusion of exon 6 and exclusion of exon 7 incorporates an in-frame stop codon Codes for a truncated isoform caspase-10C		Breast cancer	[96]
		Caspase-10D	Inclusion of exons 6 and 7 Codes for the apoptotic isoform caspase-10D		Neuroblastoma, colon, B lymphoma	[94]
		Caspase-10G	Inclusion of 96-bp sequence between exons 5 and 6 (exon 5b)—lacks catalytic domain Codes for the truncated anti-apoptotic isoform caspase-10G	Either inactive or act as a dominant-negative inhibitor of cellular apoptosis	Cervical cancer, leukemia, breast cancer	[97]
ADAMs	ADAM9	*ADAM9-L*	Full-length variant Codes for the transmembrane isoform ADAM9-L	Transmembrane protein signaling—suppresses cell-migration	Breast cancer	[98]
				Enhances cell adhesion and invasion	Lung cancer	[99]
		ADAM9-S	Inclusion of exon 12 which incorporates an in-frame stop codon. Codes for the secreted isoform ADAM9-S	Metalloprotease activities of the secreted form of the protein—induces cell migration	Hepatocellular carcinoma, colon cancer, breast cancer	[98, 100]
	ADAM12	*ADAM12-L*	Exclusion of exon 19 Codes for the transmembrane isoform ADAM12-L	Shedding EGFR ligands and cell adhesion proteins—enhances cellular proliferation, invasion, and metastasis	Small cell lung cancer, breast cancer	[101–103]
				Reduces invasion or metastasis	Breast cancer	[104]
		ADAM12-S	Only contains up to exon 19 Codes for the secreted isoform ADAM12-S	Enhances cellular proliferation, invasion, and metastasis	Lung cancer, breast cancer	[102, 105]
	ADAM15	*ADAM15-A*	Classical splice variant Codes for the classical ADAM15 isoform	Ectodomain shedding of cadherins, altering cell–cell adhesion, ECM degradation—induces cancer metastasis	Ovarian, gastric, breast, prostate, lung cancers, and brain cancer	[106–111]
		ADAM15-B	Insertion of 75-bp sequence (20a) between exons 19 and 22 Additional proline-rich insertion Codes for the ADAM15-B isoform	Interaction with extracellular signal-regulated kinase and adaptor molecules—triggers intracellular signaling pathways to induce cancer metastasis	Breast cancer	[112]
		ADAM15-C	Insertion of 72-bp sequence (20a+21) between exons 19 and 22 Additional proline-rich insertion Codes for the ADAM15-C isoform			
		ADAM15-D	Exclusion of exons 19 to 21, causing a frame shift in exons 22 and 23 and premature stop codon—lacks proline-rich modules Code for the truncated ADAM15-D isoform	Unknown function		

[22]. PSA is the most extensively studied KLK member and the most valuable biomarker in clinical medicine for prostate cancer diagnosis [118]. PSA is involved in the cleavage of semenogelins I and II, ensuing in liquefaction of the seminal coagulum after ejaculation [119]. In addition, PSA can cleave several other substrates, explaining its putative functions in modulating the prostate tumor microenvironment. For example, it was previously found that PSA can cleave components of the ECM, as well as proteins in the basement membrane, suggesting its crucial role in cancer cell invasion (Table 1; Fig. 1(a)) [18, 19]. On the contrary, a significant reduction of lung metastasis has been observed in an experimental *in vivo* metastasis assay, indicating that KLK3 can inhibit the metastatic process [20]. This anti-metastatic effect was later found attributable to anti-angiogenic activity of KLK3/PSA, which converts Lys-plasminogen to anti-angiogenic angiostatin-like fragments (Table 1; Fig. 1(a)) [21].

Up to date, fifteen *KLK3* splice variants, with eight protein isoforms, have been predicted [22]. Among these, five common KLK3 isoforms, PSA-related proteins 1 to 5 (PSA-RP1-5), were detected in normal, benign prostatic hyperplastic (BPH), and malignant prostate tissues (Table 1) [22]. The C-terminal region of PSA-RP1, PSA-RP2, and PSA-RP5 is different from the full-length PSA, whereas a frame deletion of PSA generates truncated PSA-RP3 and PSA-RP4 [22]. Heuzé et al. identified the first *KLK3* splice variant: *PSA-RP1* generated from the alternative splicing of intron 4, that codes for isoform PSA-RP1 (length, 238 aa; mass, 26.3 kDa) in prostatic LNCaP cells (Table 1; Fig. 1(b)) [23]. The serine residue in the active site is absent in the PSA-RP1 isoform, therefore compromising its catalytic activity. In addition, this alteration prevents the interaction of PSA-RP1 with the serum inhibitors required for PSA-RP1 clearance; hence, it is accumulated in serum [23]. Since, PSA-RP1 is not a serine protease, it may remain uncomplexed in the serum, and the molecular mass of the mature recombinant PSA-RP1 was found to be similar to the molecular mass of free recombinant PSA [23]. Lai et al. have demonstrated that the *KLK3* splice variant encoding PSA-RP1 is overexpressed in the LNCaP prostate cancer cell line [120]. Riegman et al. identified the second *KLK3* splice variant, *PSA-RP2*, which arises from retention of intron 3 that generates a premature stop codon, codes for isoform PSA-RP2 (length, 180 aa; mass, 25 kDa) (Table 1; Fig. 1(c)) [121]. Similar to PSA-RP1, the PSA-RP2 isoform also lacks the serine residue of the catalytic domain, hence no catalytic activity [24]. However, *PSA-RP2* expression was found comparatively higher in prostate cancer cells than in benign prostatic cells [24]. Also, the overexpression of *PSA-RP2* in PC3 prostate cancer cells slightly decreased cell proliferation and increased cell migration suggesting its functional role in prostate cancer (Table 1) [24]. Tanaka et al. identified another *KLK3* splice variant, *PSA-RP3*, which results from the alternative splicing of exon 3, excluding 129-bp region in exon 3 generating a premature stop codon. This variant codes for the truncated isoform PSA-RP3 (length, 218 aa; mass, 23.7 kDa) which lacks 44 aa residues from mature PSA (Table 1; Fig. 1(d)) [25]. This results in the deletion of asparagine-45:N-glycosylation binding site for the carbohydrate/glycan chain of PSA [25]. PSA glycosylation is involved in the conformation and folding of the PSA and affects its stability and activity [122]. Hence, the loss of a glycan chain from PSA-RP3 isoform might affect its stability and catalytic activity compared with the classical PSA isoform. *PSA-RP4* is generated from the alternative splicing of exon 3 and encodes the truncated isoform PSA-RP4 (length, 220 aa; mass, 25 kDa) with an in-frame deletion (42 aa) from its catalytic domain (Table 1; Fig. 1(e)) [22]. The isoform PSA-RP5 (length, 227 aa) is coded by the splice variant *PSA-RP5* which arises from the alternative splicing of intron 4 and exclusion of exon 5 (Table 1; Fig. 1(f)) [22]. Similar to PSA-RP1 and PSA-RP2 isoforms, PSA-RP5 also lacks the serine residue of the catalytic domain, hence no catalytic activity. Although PSA-RP1-5 isoforms were found differentially expressed in prostate cancer cell lines and patient tissues compared with normal cell lines and tissues, their specific function in prostate cancer pathology is still under investigation [22]. On the other hand, a novel *KLK3* splice variant, *PSA-SV5*, was identified by Pampalakis et al. particularly in prostate cancer (not shown in the figure) [26]. Although the isoform coded by this variant is not fully characterized, analysis of the inferred amino acid sequence using SignalP has identified a signal peptide, suggesting that the encoded protein is secreted [26]. However, the predicted PSA-SV5 isoform displays 100% identity at the C-terminal domain of classical PSA yet is predicted to lack the catalytic histidine at the catalytic domain, hence no enzymatic activity [26]. The differential expression of the *PSA-SV5* variant in BPH and prostate cancer suggests its usefulness in distinguishing normal and BPH forms from malignant prostate cancer [26]. In addition, it has been suggested that expression of *PSA-SV5* may be examined in combination with the classical *KLK3/PSA* transcript as an exclusive biomarker for prostate cancer to increase the specificity of the current PSA diagnostic test [26].

2.1.2 KLK4

Similar to *KLK3*, differential roles of *KLK4* variants and isoforms have gained an increasing interest particularly in relation to prostate cancer [123, 124]. Several alternative transcripts coding different isoforms have been reported for *KLK4* [28, 29, 125–128]. The classical full-length catalytic isoform KLK4 (length, 254 aa; mass, 27 kDa) was found to be associated with enhanced proliferation, colony formation, and migration in prostate cancer cells (Table 1) [129, 130]. KLK4 contributes to degenerating ECM by activating the uPA–uPAR system and liberating pro-angiogenic growth factors [27]. Furthermore, tumor invasion and migration can be

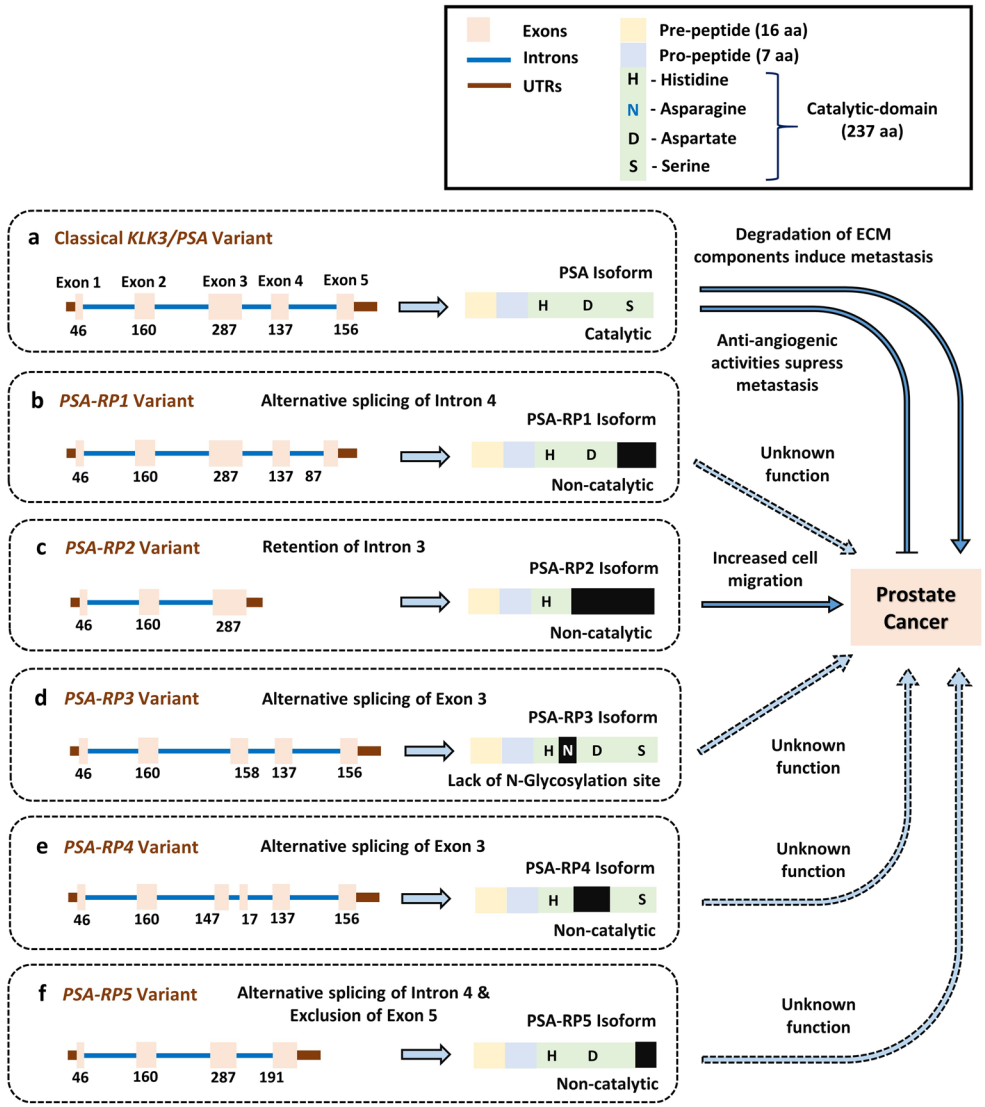

Fig. 1 A schematic representation of the *KLK3* splice variants and isoforms and their distinct roles in prostate cancer. (a) Classical *KLK3/PSA* variant codes for the full-length catalytic PSA isoform. PSA is involved with the degradation of ECM components inducing metastasis. Also, PSA can activate anti-angiogenic signaling which in turn suppresses tumor metastasis. (b) *PSA-RP1* variant is generated from the alternative splicing of intron 4 and codes for the PSA-RP1 isoform, lacking the serine residue in the catalytic domain. (c) *PSA-RP2* is generated from the retention of intron 3 excluding exons 4 and 5 and codes for the PSA-RP2 isoform, lacking the serine residue of the catalytic domain. PSA-RP2 increases cancer cell migration. (d) *PSA-RP3* is generated from the alternative splicing of exon 3 and codes for the PSA-RP3 isoform, lacking the asparagine-45 glycosylation site. (e) *PSA-RP4* is generated from the alternative splicing of exon 3 and codes for the PSA-RP4 isoform, lacking 42 aa region from the catalytic domain. (f) *PSA-RP5* is generated from the alternative splicing of intron 4 and exclusion of exon 5 and codes for the PSA-RP5 isoform, lacking the serine residue of the catalytic domain. All *PSA-RP1-5* splice variants produce truncated non-catalytic isoforms. Cancer-specific functions of PSA-RP1, PSA-RP3, PSA-RP4, and PSA-RP5 isoforms in prostate cancer progression are still unknown. Highlighted black box: regions deleted in each KLK3 isoform

facilitated by KLK4-mediated pro-MMP activation [27]. In addition, KLK4 can cleave PAR-1 and release interleukin-6 (IL-6), to mediate tumor–stroma interactions for local invasion [131]. Along with the full-length KLK4 isoform, two truncated isoforms were identified, which were abundantly expressed in prostate cancer [28]. The *KLK4-205* variant, which originates from the exclusion of exon 1, codes for an N-terminally truncated isoform KLK4-205 (length, 205 aa; mass, 29 kDa). This isoform is unlikely to be secreted, since it lacks the signal peptide and pro-region of the pro-enzyme (Table 1) [28]. The *KLK4-IRIII* variant which results from the retention of intron 3 creates a unique C-terminal end containing a premature stop codon, hence predicted to code for a truncated protein (Table 1) [30]. The prevailing signal peptide suggests that it can be secreted but will not function as a serine protease as it lacks the serine residue in the catalytic domain [30]. Dong et al. have demonstrated that full-length KLK4 isoform was detected mainly in the cytoplasm, while the

truncated KLK4-205 isoform is in the nucleus of PC3 prostate cancer cells [28]. Differential cellular localization of these truncated isoforms suggests they might have differential roles in prostate cancer progression. However, the individual role of these truncated isoforms in cancer progression remains unclear. Apart from prostate cancer, expression of the *KLK4-205* variant was reported in the OVCAR-3 ovarian cancer cell line and HEC1A/B, ISH, KLE, RL endometrial cancer cell lines [29, 127].

2.1.3 KLK6

KLK6 (encoded by the gene *KLK6*) was earlier suggested to be a tumor suppressor [132]. Later, it was found to be differentially expressed in a wide range of cancers such as colon, ovarian, lung, and gastric, where its upregulation was associated with cancer invasion and metastasis and reduced patient survival [31, 133, 134]. Eleven splice variants designated A to E and v6 to v11 of *KLK6* that encode eight different protein isoforms have been identified in human cancer cell lines [135]. Splice variants *KLK6-A* and *KLK6-B*, which differ in the length of their 5′ untranslated region (UTR), code for the full-length catalytic isoform KLK6 (length, 244 aa; mass, 26.8 kDa) (Table 1) [135]. KLK6 was found to be associated with increased metastasis via KLK6-mediated E-cadherin shedding, thereby disturbing E-cadherin-mediated cellular adhesion [32, 133]. Moreover, higher expression of KLK6 was found in breast cancer, melanoma, and lung cancer, leading to invasiveness of tumor cells by degrading ECM components [33]. Adamopoulos et al. described the complete repertoire of *KLK6* variants showing that splice variants *KLK6-C*, *KLK6-D*, and *KLK6-E* code for a series of N-terminally truncated isoforms [135]. These truncated isoforms, which lack the signal peptide and glycosylation sites, are not secreted and unable to undergo catalytic activation (Table 1) [136]. Although, the cancer-specific expression of all *KLK6* variants has been widely studied at the mRNA level, expression of the truncated isoforms and their potential functional role in cancer needs further investigation [135].

2.1.4 KLK8

Altered expression of KLK8 was reported in various human cancers including lung, head and neck, uterine endometrial, cervical, salivary gland, colorectal, and ovarian cancers [137–142]. In addition to the full-length *KLK8* variant, several other *KLK8* splice variants and their isoforms were detected in these malignancies [137–142]. *KLK8-v1* and *KLK8-v2* splice variants consist of 6 exons and code for two catalytic KLK8 isoforms, KLK8-1 (length, 260 aa; mass, 28 kDa) and KLK8-2 (length, 305 aa; mass, 32.9 kDa), respectively (Table 1) [135]. Since these two isoforms harbor two pro-enzymes that differ in pro-peptide sequences, they have cell type–dependent secretion and are thought to be activated differently [143]. Sher et al. showed that over expression of full-length *KLK8* reduces cancer cell migration and invasion by inhibiting the binding of fibronectin and integrin, thereby downregulating vascular endothelial growth factor (VEGF) signaling [35]. In addition to the above two splice variants, three other *KLK8* splice variants were identified: *KLK8-v3*, which lacks exons 3 and 4; *KLK8-v4*, which lacks exons 3, 4, and 5; and *KLK8-v5*, which lacks exon 3 and has a new coding starting site at exon 4 (Table 1) [36, 143]. These variants code for 3 different truncated isoforms (length, 119 aa, 32 aa, and 139 aa, respectively) which lack a part of the catalytic domain and the signal peptide, hence no activation or secretion [36]. Similar to other KLKs, the individual roles of KLK8-truncated isoforms still remains unclear. Yet, several studies report the upregulated expression of *KLK8-v3-5* splice variants in a wide-range of cancer cell lines and tissues, such as in lung, breast, ovarian, endometrial, and prostate cancers [65–67].

Although, full-length KLK isoforms show context-dependent tumor-promoting or tumor-inhibiting effects via their serine-protease activities, many truncated KLK isoforms are unable to be secreted or catalytically activated. Nevertheless, the differential expression of these truncated KLK isoforms in cancers may suggest other cancer-specific functions, albeit not yet proven. It is clear that cancer cells have differential compositions of the *KLK* variants and isoforms, and quantification of such *KLK* variants in human samples might provide clinical applications in a wide range of cancers.

2.2 Cathepsins

Cathepsins are comprised of 12 protease members (11 papain-like cysteine cathepsins and 1 aspartyl cathepsin). Upregulated expression of cathepsins are often found correlated with the metastasis of various cancers, such as breast (cathepsin D, B, H, L), head and neck (cathepsin B, H, L), colorectal (cathepsin B), and prostate cancer (cathepsin B, S) [144, 145]. They have multiple roles during tumorigenic processes including cell proliferation, angiogenesis, metastasis, and invasion, while being secreted into the extracellular milieu [145]. Via pharmacological inhibition of cathepsins in animal models, investigators have established the causative relationships between cancer progression and deregulation of cysteine cathepsins [146]. Below we describe some examples of cathepsins in cancer, which are regulated by alternative splicing machinery.

2.2.1 Cathepsin B

Cathepsin B (encoded by the gene *CTSB*) is often linked to multiple stages of cancer progression; hence, it is considered a potential therapeutic target for many cancers [147].

Particularly, both *in vivo* and *in vitro* models have been used to demonstrate the crucial role of cathepsin B in metastasis and invasion of various tumors, including melanomas, breast, lung, cervical, and glioblastoma [38–48, 147]. Proteolytic activity of cathepsin B is necessary for fibroblast-mediated invasion, and it can shape the tumor microenvironment by (i) ECM remodeling and degradation by their extracellular functions and (ii) by activating caspase-cascades which trigger degradation of ECM components [47, 148–151]. *CTSB* pre-mRNA is comprised of 12 exons with 11 introns [16]. It has been found that a partial Alu element is present in exon 2 of the *CTSB* gene, which may influence it's translational regulation [152]. In addition, these interspersed Alu elements may allow for additional alternative splicing in exon 2, which in turn increases the diversity of *CTSB* transcripts [152]. Although, many *CTSB* splice variants and potential protein isoforms were recorded, two splice variants were commonly found with altered levels of expression in cancer [153]. Gong et al. described the *CTSB* splice variant which lacks exon 2; *CB* (*-2*) codes for the catalytic isoform CB (-2) (length, 339 aa; mass, 37.8 kDa) which is translated twice as efficiently compared with the unspliced variant (Table 1) [16]. Consistently, higher expression of the *CB* (*-2*) splice variant was observed in many cancer cell lines, including breast and colon (metastasis to liver) [16]. Another splice variant, *CB* (*-2*, *3*), in which both exons 2 and 3 have been spliced-out, was identified that produces a truncated isoform tCB (mass, 32 kDa) (Table 1) [16]. Isoform tCB lacks 17 aa from the signal peptide and the first 34 aa from the pro-peptide. Increased expression of the *CB* (*-2*, *3*) variant was found in many cancer cell lines, including human melanomas: A375P (parental, poorly metastatic) and A375M (metastatic), breast, and colon (metastasis to liver) cell lines [16, 49]. Gong et al. described that the *CB* (*-2*, *3*) variant is translated nearly four times more efficiently than the *CB* (*-2*) variant, and twice the rate as the unspliced variant [16]. It is hypothesized that tCB would not be targeted to either ER or lysosome pathways as it lacks the signal peptide [16]. Later, it was found that tCB is misrouted from its regular biosynthetic pathway and forced to enter into its final destination: mitochondria (Fig. 2) [153, 154]. This property of tCB might be due to the incorporation of a latent mitochondrial import leader sequence in the N-terminus end of the isoform [154]. Mehtani et al. reported that the protein product of the *CB* (*-2*, *3*) variant can fold properly and show active proteolytic activities *in vitro* [49]. In addition, the above study found the tCB isoform to be located on the cytosolic surface of intracellular membranes. After localization to the mitochondria, tCB is expected to interact with key regulatory proteins such as Bcl-2 and Ras-related proteins, which are associated with diverse biological functions (Fig. 2) [155–157]. However, contradictory results were given by Müntener et al. proposing that tCB is catalytically inactive and cannot properly fold into an active protease [158]. This study suggested that any pathophysiological involvement of tCB must be attributed to properties other than their proteolytic activities. Both Müntener et al. and Bestvater et al. reported that the naturally occurring tCB was directed to the mitochondria and that the cells died after nuclear fragmentation (Fig. 2) [154, 159]. Hence, it is clear that tCB might be involved in regulating cell apoptosis via its non-proteolytic activities. However, it is not yet clear whether it promotes malignancies, or it has any tumor-suppressor functions, even though its expression was observed in many cancers. Therefore, further *in vitro* and *in vivo* investigations are required to confirm the specific function of tCB in malignancies.

2.2.2 Cathepsin L

Cathepsin L (encoded by the gene *CTSL*) is a lysosomal endopeptidase which was found overexpressed in number of human cancers [160]. Numerous studies showed that cathepsin L upregulation is a critical feature in triggering the switch of non-metastatic human cancers to a highly aggressive metastatic phenotype [50, 161]. Cathepsin L was found to be involved in antigen processing, bone resorption, turnover of intracellular proteins involved in regulation of growth, and degradation of ECM components triggering cancer cell invasion and metastasis [162, 163]. Cathepsin L can also augment the proteolytic cascade by triggering latent pro-forms of other proteases, such as proteases involved in tumor metastasis [164]. Although only one catalytic isoform of cathepsin L was identified up to date, a number of *CTSL* splice variants with differential translation efficiencies were identified in many cancers [50, 53, 55, 165]. The *CTSL* gene produces two types of pre-mRNAs: *hCATL-A* and *hCATL-B*, which code for the full-length catalytic isoform cathepsin L (length, 333 aa; mass, 37.5 kDa) [51, 52, 165]. Four splice variants of *hCATL-A* have been identified: *L-A*, *L-A1*, *L-A2*, and *L-A3*, which differ from each other by the length of 5′ UTR (Table 1) [52, 165]. Abudula et al. showed that the shortest variant, *L-A3*, is common in both normal and several cancerous tissues (kidney, breast) and found translated two to three times more efficiently compared with other variants [53]. Similarly, efficient translation of *L-AII* (homologous to *L-A2*) and *L-AIII* (homologous to *L-A3* but has two additional GC nucleotides) variants has been demonstrated in five distinct cancer cell lines [52]. In addition, Caserman et al. have observed a marked expression of the *L-A3* variant in pre-cancerous breast epithelial and carcinoma cell lines compared with the *L-A2* variant [165]. Moreover, Mittal et al. have revealed that the longest variant, *L-A*, exhibits a higher translatability in cancer cells notwithstanding its lower stability compared with the shortest variant, *L-AIII* [166]. Most cellular mRNAs were found with an active internal ribosomal entry site (IRES) in the 5′ UTR, which enables cap-independent translation initiation under various stress conditions [167].

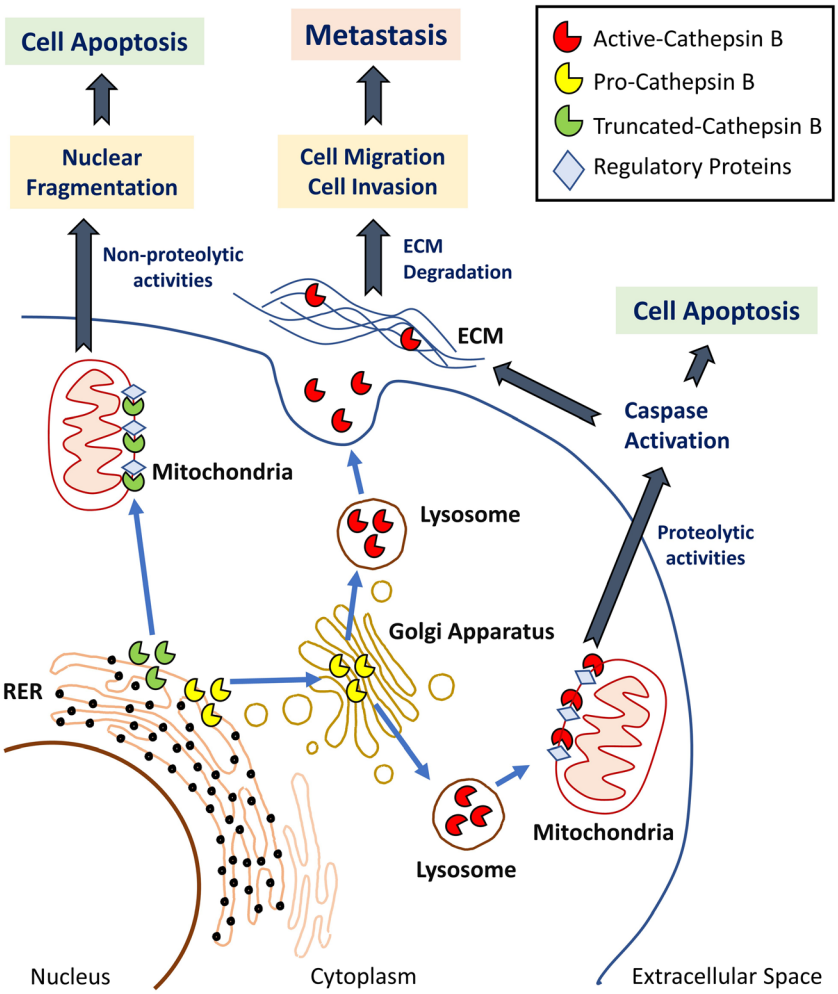

Fig. 2 Proposed roles of *CTSB* variants in the tumor microenvironment driven through distinct molecular trafficking mechanisms and opposing proteolytic activities. Alternative splicing of cathepsin B pre-mRNA produces two major splicing variants on the RER, where they are translated and posttranslational modifications are carried out. The *CB* (*-2*) variant translates into the pre-pro-enzyme which will next move into the lumen of RER where the inactive precursor form: pro-cathepsin B (yellow symbol) is formed. Pro-cathepsin B is then transported through the RER to the Golgi apparatus and converted to active-cathepsin B (red symbol) by autocatalytic activation as a result of proteolytic cleavage and dissociation of the pro-peptide. Secreted active proteolytic cathepsin B can be involved in the remodeling and degradation of ECM components by its extracellular functions and intracellularly by activating caspase-cascades which trigger both cell apoptosis and ECM degradation. ECM remodeling and degradation facilitate tumor cell migration and invasion leading to cancer metastasis. In contrast, the truncated cathepsin B which is translated by the *CB* (*-2*, *3*) variant does not transport into the endolysosomal compartment but is localized to mitochondria. Possible interaction between truncated cathepsin B and regulatory proteins on mitochondria could trigger nuclear fragmentation and subsequent cell apoptosis via its non-proteolytic activities. RER, rough endoplasmic reticulum; ECM, extracellular matrix

Accordingly, previous studies have suggested the presence of functional IRESs in the *L-A* variant might contribute to its increased translation in cancer cells [166, 168]. However, Tholen et al. later described that the *L-A3* variant can mediate stress-resistant translation in breast cancer cell lines, although it does not contain IRES motifs to enhance translation initiation [50]. In addition, a *CTSL* variant overexpressed without its UTRs (containing only the coding sequence) was found to be sensitive to stress-induced translational shutdown. Therefore, it can be hypothesized that the stress-resistant translation of *CTSL* variants is facilitated only by the UTR (independent from IRES motifs), which helps *CTSL* variants to escape being deposited in translationally inactive stress granules. This post-transcriptional mechanism is hence suggested to be involved in promoting cathepsin L–mediated tumor progression and metastasis. Pre-mRNA *hCATL-B* produces only one splice variant, *L-B*, which is transcribed from another TATA-less promoter localized within intron 1 (Table 1) [51, 53, 55]. It was found that *L-B* differs from *hCATL-A* variants only in the length of 5′ UTR. Caserman et al. showed that expression of the *L-B* variant in breast cancer cell lines was low or negligible. However, Chauhan et al. reported that the *L-B* variant was highly abundant in human adenocarcinoma, hepatoma, and renal cancer cell lines [55]. Accordingly, it is clear that switching from one *CTSL* variant to another creates an opportunity for the cancer cell to regulate cathepsin

L levels within the tumor microenvironment. Predominance of efficiently translated splice variants in cancer cells proposes that such upregulated splice variants play a key role in the overexpression of cathepsin L in cancer.

Upregulated expression of cathepsins are documented at the mRNA and protein levels in many cancers, as described above for cathepsins B and L. Studies which define the cathepsin cellular localization and interactions with other pathways suggest the possibility that cathepsin isoforms and associated pathways can be targeted in cancer therapeutics [16]. However, whether these dynamic roles of cathepsin isoforms come from tumor cells, tumor-associated cells, or both are still unknown. Importantly, as previously suggested, such upregulated expression of cancer-specific cathepsin isoforms at the tumor cell membranes could be used as prodrug activators in targeted cancer therapy [169].

2.3 Caspases

Caspases are cysteinyl aspartate proteinases which are involved in cell regulatory networks controlling inflammation and both intrinsic and extrinsic apoptotic pathways [170–172]. Dysregulation of caspase activities and failure to exclude damaged cells by caspase-mediated cellular apoptosis can cause neoplastic cell growth, prompting both tumorigenesis and cancer progression [173]. Caspases are responsible for modulating the turnover of cell adhesion molecules by modifying intercellular attachments and degrading the ECM by indirectly affecting the secretion of inflammatory factors and MMPs [174, 175]. Therefore, caspases play a key role in regulating cellular motility in normal and metastatic cancer cells [176]. Similar to other proteases, caspases are expressed as pre-pro-enzymes. All caspases contain two essential caspase catalytic domains (p10 and p20) containing the key catalytic residues (cysteine and histidine). Some caspases contain only the catalytic domains (e.g., capcases 3, 6, 7, 14), while initiator caspases contain an additional tandem pair of death effector domain (DED) (e.g., caspases 8 and 10). Moreover, some initiator caspases and caspases which are involved in intrinsic pathways contain caspase recruitment domains (CARD) (e.g., caspases 1, 2, 4, 5, and 9) [177]. Both the DED and CARD motifs mediate specific protein–protein interactions. Alternative splicing events in caspase pre-mRNAs incorporates premature stop codons or deletions in the transcript variant coding for truncated isoforms lacking the catalytic domain, DED or CARD domains [63, 67, 93, 94, 178]. Such truncated isoforms were often found as endogenous inhibitors of the cell apoptotic pathways associated with a wide variety of tumors (Table 1) [17, 53, 63, 67, 93, 94, 179]. Therefore, alternative splicing of caspase pre-mRNA is considered to be crucial for determining cancer cell susceptibility or resistance to apoptosis.

2.3.1 Caspase 2

Caspase 2 (encoded by the gene *CASP2*) is one of the most highly conserved members of the caspase family, which is activated by various stimuli, including chemotherapeutic agents, oxidative agents, and stress signals [172, 180]. Given the essential role of caspase 2 as a regulator of apoptosis initiation, caspase 2 has been proposed to possess tumor suppressor functions. Somatic mutations and reduced caspase 2 expression were often found to be associated with poor prognosis of various cancers, including gastric, colorectal, breast, and ovarian cancers, leukemia, and glioblastomas [56–60]. Alternative splicing of *CASP2* pre-mRNA generates two caspase 2 isoforms with distinct functions in apoptosis [17]. Exclusion of exon 9 results in the *CASP2* long variant, *caspase-2L*, which translates into the isoform caspase-2L (length, 452 aa; mass, 50.6 kDa), which shows apoptotic functions (Table 1; Fig. 3(a)) [63]. The inclusion of exon 9 incorporates a premature stop codon in the *CASP2* short variant; *caspase-2s*, producing a truncated isoform caspase-2s (length, 313 aa; mass, 34.9 kDa) which lacks the small subunit of the catalytic domain and inhibits apoptosis (Table 1; Fig. 3(a)). Caspase-2L is expressed in a wide range of tissues, whereas caspase-2s was found primarily expressed in the brain, heart, and skeletal muscle [181, 182]. Droin and colleagues described the heterogeneous expression of caspase-2L and caspase-2s isoforms in acute myelogenous leukemia [61]. Association of the RNA binding motif 5 (RBMP) protein with *CASP2* pre-mRNA intronic sequences were found in regulating the ratio of caspase-2L/2s isoforms, suggesting that *CASP2* splicing regulation by RBM5 may contribute to its tumor suppressor activity [183]. On the other hand, Droin et al. established that *caspase-2s* overexpression can selectively inhibit chromatin condensation, apoptotic body formation, membrane blebbing (protrusion of the plasma membrane), and phosphatidylserine externalization in the human leukemic cell lines [184]. Also, caspase-2s was found to antagonize apoptosis by preventing caspase-2L and caspase 3 activation and preventing apoptotic blebbing and body formation in human B lymphoma [64, 185]. Caspase-2s was shown to be responsible for inhibiting DNA damage–induced cytoplasmic cleavage of cytoskeleton proteins (Fodrin) and averting cisplatin-induced membrane blebbing in ovarian cancer [63]. The same study confirmed the expression of caspase-2s in various cancers, such as cervical and lung cancers, yet it was undetectable in colorectal cancer and glioblastoma. Interestingly, Logatte et al. described that the ratio of *caspase-2L*/*2s* mRNA is maintained by the promoter strength, alternative transcriptional initiation, and 5′-splicing events [186]. The study demonstrated that the *caspase-2L* promoter is much stronger compared with the *caspase-2s* promoter, in good agreement with the respective transcript levels. High *caspase-2L*/*2s* ratio (approximately 100-fold) was found in several cell lines including leukemia,

carcinoma, and immortalized cells [187]. However, *caspase-2S* mRNA was shown to be very short-lived and may thus normally not be expressed or only at very low levels [187]. Low levels of *caspase-2S* can be explained by nonsense-mediated mRNA decay (NMD), an active mRNA degradation mechanism which reduces errors in gene expression by removing transcripts that contain premature stop codons [188].

In addition to *caspase-2L/-2s* splice variants, Droin et al. found two additional *CASP2* mRNAs, termed as *CASP-2l-Pro* and *CASP-2s-Pro* [62]. The isoforms coded by these mRNAs, caspase-2l-Pro and caspase-2s-Pro, corresponded to the pro-domain of pro-caspase-2L and pro-caspase-2s [62]. Alternative splicing creates a translational frameshift with a premature stop codon in *CASP-2l-Pro* and codes for the caspase-2l-Pro isoform (length, 91 aa) in which the last α-helix of its CARD is deleted. The study showed that the caspase-2L-Pro isoform negatively interferes with pro-caspase-2L activation and death receptor- and drug-mediated apoptosis in normal and cancer cell lines (human Burkitt lymphoma cell line) (Table 1) [62].

2.3.2 Caspase 3

Caspase 3 (encoded by the gene *CASP3*) is associated with the process of physiological cell migration and cancer cell metastasis in a context-dependent manner. It has been reported that caspase 3 controls the cleavage of neuronal microtubule-stabilizing proteins which assist in cytoskeleton disassembly, enabling the dispersion of neuronal cells [189]. In addition, pro-caspase 3 can regulate fibronectin secretion, which affects cell adhesion, migration, and survival [190]. In metastatic scenarios, basal caspase 3 activity is reported to increase migration and invasiveness in glioblastoma cells [191]. Also, caspase 3 activation was found to be linked with migration and invasion in ovarian, melanoma, and hepatoma cancer cells [68, 69, 73]. Human *CASP3* generates two major isoforms, with distinct apoptotic activities [67]. The longest mRNA variant, *caspase-3L*, produces the principal isoform caspase-3L (length, 277 aa; mass, 31.6 kDa) which demonstrates apoptotic functioning (Table 1; Fig. 3(b)). Huang et al. characterized the second *CASP3* variant, *caspase-3s*, which excludes the exon 6 sequence and translates into an anti-apoptotic short-isoform caspase-3s (length, 277 aa; mass, 20 kDa) lacking part of the short subunit of the catalytic domain (Table 1; Fig. 3(b)) [67]. The same study reported the expression of caspase-3s in normal and cancer cell lines (cervical cancer, neuroblastoma) [67]. However, endogenous caspase-3s levels were often lower compared with caspase-3L levels in cancer cells; hence, caspase-3s levels were likely not sufficient to completely suppress apoptosis [67]. Additionally, results of the above study uncovered an additional role of the proteasome in caspase-3s degradation. However, the study suggests that under certain chronic pathophysiological conditions such as carcinogenesis, an alteration of splicing events may create a discrepancy in caspase-3L/3s ratios in tumor cells, altering the cells' susceptibility to apoptosis [67].

2.3.3 Caspase 8

Loss of heterozygosity in the region containing the *CASP8* gene is associated with many types of tumors [192]. Some studies have reported crosstalk between caspase 8 and cytoskeleton remodeling that plays a crucial role in the modulation of cell adhesion and cell migration in cancer metastasis [193, 194]. Although the deletion of the *CASP8* gene had no effect on primary tumor development, loss of caspase 8 was found to be associated with high-risk metastatic neuroblastoma due to impaired programmed cell death [195, 196]. In contrast, some other studies showed that *CASP8* gene deletion is associated with lowering the incidence of distant metastasis [74, 75, 78]. It has been shown that caspase 8 expression sustains NF-κB nuclear accumulation and correlates with NF-κB transcriptional activation in glioblastoma cell lines. While a number of caspase 8 isoforms are described (caspase-8a-h), only two pro-apoptotic isoforms are identified in many tissues and cell lines: caspase-8a (length, 496 aa; mass, 55.4 kDA) and caspase-8b (length, 479 aa; mass, 53.7 kDA) which differ only in a short sequence within the pro-peptide that derives from alternative splicing of exon 8 (Table 1; Fig. 3(c)) [83, 178, 197, 198]. These two isoforms show ubiquitous expression at equivalent levels, and function interchangeably [197]. Horiuchi et al. have described the predominant expression of the anti-apoptotic isoform caspase-8L (length, 538 aa; mass, 32.3 kDA) lacking catalytic domains [178]. The *caspase-8L* splice variant is produced by the alternative splicing of intron 8, thereby carrying a 136-bp insertion (exon 8b) and frame shift of the transcript (Table 1; Fig. 3(c)) [178]. It has been reported that caspase-8L is associated with undifferentiated neuroblastoma and stem cell–derived leukemia by providing protection from caspase 8–dependent apoptosis [79, 80]. Furthermore, an association between the rarest caspase 8 isoform caspase-8h (length, 496 aa; mass, 57.7 kDA) and cell motility was suggested by previous ectopic expression studies [81, 82, 199]. The second DED of this isoform is disrupted by insertion of a 17 aa sequence containing two tryptophan residues [194, 198]. It has been suggested that this unusual pro-peptide region in the caspase-8h isoform associates with tyrosine kinase Src and facilitates Src-mediated motility in neuroblastoma cells [82]. Xu et al. have reported the novel short isoform caspase-8s (length, 108 aa; mass, 13 kDa) with a 106-bp deletion, resulting in a frameshift mutation carrying a premature stop codon [83]. This variant codes for the caspase-8s isoform which includes the

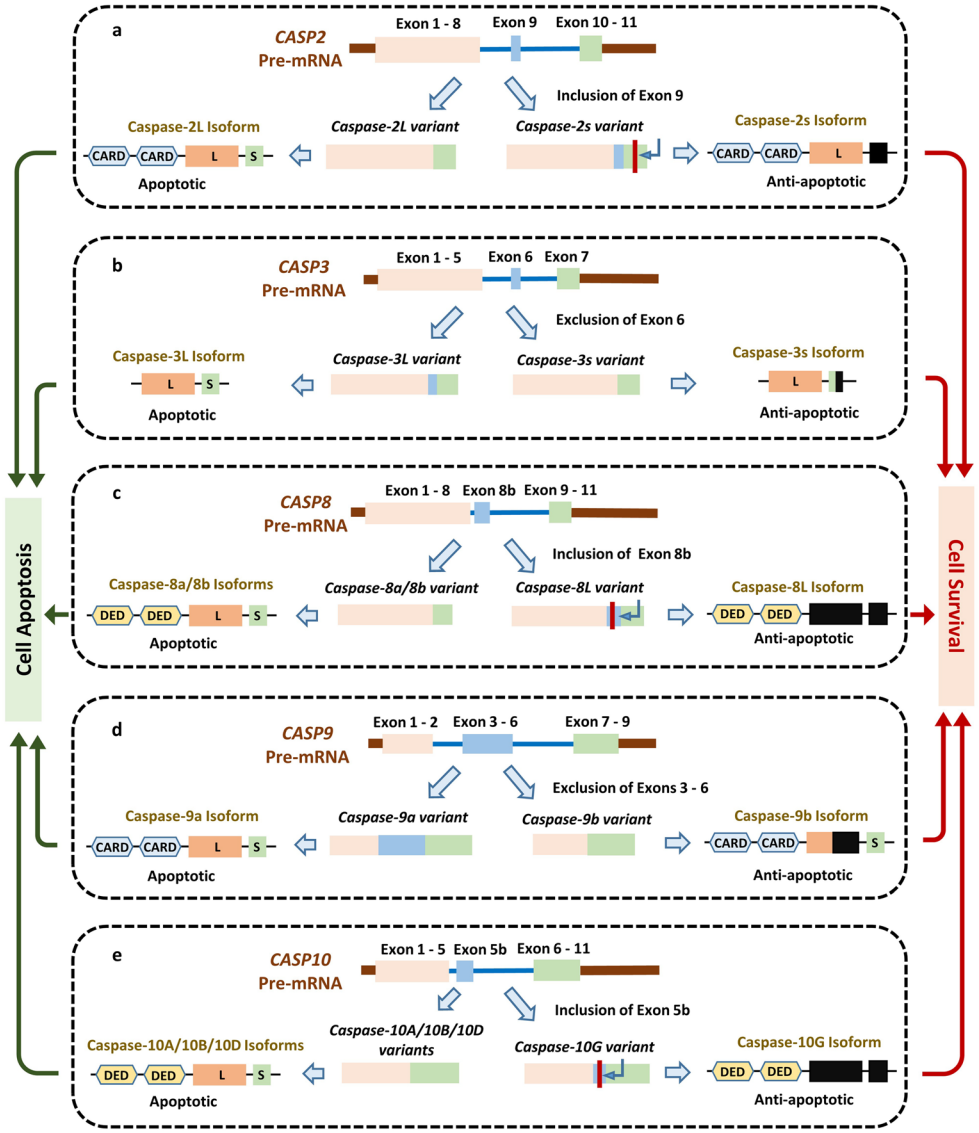

Fig. 3 A schematic representation of the genomic structures of human caspase variants characterized according to their opposing activities between cell apoptosis and cell survival. Exon arrangement of apoptotic and anti-apoptotic isoforms of caspases 2, 3, 8, 9, and 10 is demonstrated. Left panel of each caspase illustrates the apoptotic variants inducing cellular apoptosis, whereas the right panel illustrates the anti-apoptotic variant providing survival benefit against cell apoptosis machinery. (a) Inclusion of exon 9 incorporates a premature stop codon in the *caspase-2s* mRNA, producing the caspase-2s isoform, lacking the small catalytic subunit. (b) Exclusion of exon 6 sequence in *caspase-3s* mRNA translates into the caspase-3s isoform, lacking a part of the small catalytic subunit. (c) Caspase-8L isoform lacking the complete catalytic domain is generated by the inclusion of an extension of exon 8 (8b) in *caspase-8L* variant which incorporates a frame shift in the transcript. (d) Caspase-9b isoform is coded by the *caspase-9b* variant excluding exons 3, 4, 5, and 6, lacking a part of large catalytic domain. (e) Inclusion of 96-bp sequence between exons 5 and 6 (exon 5b) in *caspase-10G* variant produces caspase-10G with a premature stop codon and codes for the caspase-10G isoform, lacking the catalytic domain. Red bar: position of the premature stop codon. L, large catalytic subunit; S, small catalytic subunit; CARD, caspase recruitment domain; DED, death effector domain. Highlighted black box: regions deleted in each anti-apoptotic isoform

first DED, part of the second DED but missing the C-terminal caspase domain. The study suggests that caspase-8s may act as a promoter of apoptosis through binding to Fas-associated death domain protein (FADD) via its DED domains. Furthermore, overexpression of caspase-8s was found to be associated with increased sensitivity to the apoptotic stimuli in Jurkat acute T cell leukemia cell line [83].

2.3.4 Caspase 9

Caspase 9 (encoded by the gene *CASP9*) is a key caspase involved in the intrinsic apoptosis pathway and is activated at multi-protein activation platforms [200]. Clinical significance of caspase 9 is evidenced by the fact that functional polymorphisms in the *CASP9* gene, which leads to disruption of the intrinsic apoptosis pathway, have been shown to

participate in tumor susceptibility to a wide range of cancers [85, 89, 201]. Accordingly, previous studies confirmed the tumor suppressor function of caspase 9 in cancers [202, 203]. Two functionally distinct isoforms are produced by the alternative splicing of *CASP9* pre-mRNA [204]. First, is the larger pro-apoptotic isoform caspase-9a (length, 416 aa; mass, 46.2 kDa) that is encoded by the transcript variant, *caspase-9a*, containing all 9 exons (Table 1; Fig. 3(d)). Second, is the shorter isoform caspase-9b/s (length, 266 aa; mass, 30.1 kDA) which is encoded by the variant, *caspase-9b*, excluding exons 3, 4, 5, and 6 (Table 1; Fig. 3(d)) [87]. The caspase-9b isoform is short of the central catalytic domain and shows opposite effects by competing with its larger caspase-9a isoform for binding to the apoptosome and inducing resistance to many apoptotic stimuli [93, 205, 206]. Chalfant and colleagues revealed that endogenous generation of ceramide via *de novo* pathway and serine/arginine rich splicing factor 1 (SRSF1) results in an elevation of caspase-9a/9b ratio in A549 lung adenocarcinoma cells with a concomitant loss in the *caspase-9b* splice variant [84]. This study proposed a role for ceramide-induced alternative splicing in sensitizing cells to apoptosis. It has been shown that the *caspase-9a/9b* ratio is lower in non-small cell lung cancer (NSCLC) cell tumors which is correlated with an anti-apoptotic phenotype [92].

2.3.5 Caspase 10

The protein sequences of human caspase 10 and caspase 8 are highly homologous with their genes found in the same chromosomal region [192, 198]. However, it has been demonstrated that each caspase can activate their apoptotic pathways independently of the other [207]. It has been proposed that caspase 10 (encoded by the gene *CASP10*) has tumor–suppressor functions as acquired mutations which inactivate caspase 10 were identified in non-Hodgkin's lymphomas and solid tumor cells [208–211]. In support of this, caspase 10 expression was significantly reduced in lung and breast cancer cell lines, indicating possible selective pressure against caspase 10 expression in cancer [207]. Currently, seven *CASP10* splice variants have been reported [153, 154, 198]. However, caspase 10 is predominantly expressed as three full-length apoptotic isoform caspase-10A (length, 521 aa; mass, 58.9 kDa), caspase-10B (length, 479 aa; mass, 54.5 kDa), and caspase-10D (length, 522 aa; mass, 58.9 kDa) (Table 1; Fig. 3(e)). *CASP10* splice variants which code for the above isoforms differ according to the alternative splicing of exons 6–11 (Table 1) [96]. In addition, a splice variant with a 96-bp inclusion between exons 5 and 6 produces a truncated anti-apoptotic isoform caspase-10G (length, 247 aa; mass, 28.3 kDa), consisting of only the two DEDs without the catalytic domain [96, 97] (Table 1; Fig. 3(e)). The smaller isoform caspase-10C (length, 273 aa; mass, 31.4 kDa) is thought to be untranslated because of the nonsense-mediated mRNA decay of its transcript [97]. Mu¨hlethaler-Mottet et al. have verified that caspase-10A- and caspase-10D-mediated apoptotic pathways were strongly increased, whereas caspase-10B or caspase-10G had no effect or showed weakly anti-apoptotic activities in various caspase 8–expressing tumor cells [94]. They reported that the unique C-terminal end of caspase-10B was responsible for its degradation by the ubiquitin proteasome pathway and for its lack of pro-apoptotic activity, compared with other apoptotic isoforms. However, caspase-10B was previously demonstrated to have pro-apoptotic activities in caspase-3-overexpressing breast cancer cell lines [95]. Nevertheless, Mu¨hlethaler-Mottet et al. suggested that caspase-10G may be either inactive or act as a dominant-negative inhibitor of apoptosis, depending on the cellular context. Caspase-10G was identified to be expressed in most normal tissues with high expression in the lung, stomach, uterus, spleen, ovary, and small intestine and in cervical, leukemia, and breast cancer cell lines [97]. One recent study reported that caspase 10 can negatively modulate caspase 8 activation in response to death receptor stimulation in HeLa cells, promoting the activation of the pro-survival NF-κB-dependent cascade [212]. In addition, it was previously confirmed that overexpression of *caspase-10G* activates the NF-κB pathway in a dose- and time-dependent manner [97]. Hence, it can be hypothesized that caspase-10G also modulates the tumor microenvironment by activating the NF-kB pathway, similar to caspase 8 [213].

The above studies have highlighted the identification of truncated isoforms of caspases which act as endogenous inhibitors of cell apoptotic programming, independent of their caspase-cascade pathways. These anti-apoptotic isoforms were found naturally co-expressed with apoptotic isoforms in both normal and cancer cell lines. Since, their altered expression was found to be correlated with various hallmarks of cancer, continuing research in terms of their cellular localization and function has become potentially important.

2.4 Metalloproteases—a disintegrin and metalloproteases

A disintegrin and metalloproteases (ADAMs) belong to the MMP family and are characterized as multi-domain, single-pass transmembrane proteins that secrete metalloendopeptidases which in turn shed ectodomain proteins of the membrane-bound molecules [214]. Transmembrane ADAM proteins are composed of a pro-domain (removed in the Golgi), a metalloprotease domain (corresponding to its catalytic site), a disintegrin domain, a cysteine-rich domain, an epidermal growth factor (EGF)-like domain (stalk domain), a transmembrane domain, and a short C-terminal cytoplasmic domain (cytoplasmic tail) (Fig. 4) [215]. Several ADAMs contribute to pathogenesis of cancer, invasion, and metastasis and alter cellular signaling pathways

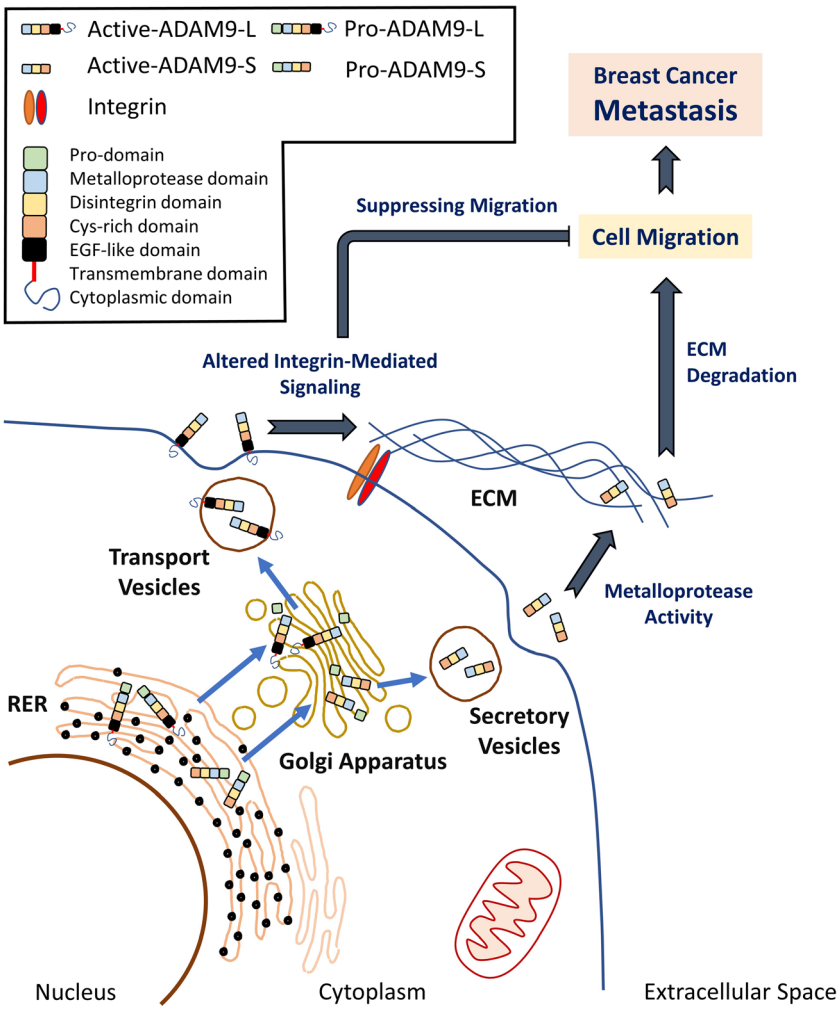

Fig. 4 Proposed molecular trafficking and opposing roles of ADAM9 protease isoforms in the breast cancer tumor microenvironment. Alternative splicing of ADAM9 pre-mRNA produces two major splice variants, which are translated into two pro-ADAM9 isoforms on RER. These pro-isoforms are then transported into the Golgi apparatus and are activated to active-ADAM9-L/S isoforms by the removal of the pro-domain. Transport Golgi vesicles carry active-ADAM9-L into the cytoplasmic membrane, while secretory Golgi vesicles aid active-ADAM9-S to be secreted out of the cell. Transmembrane ADAM9-L binds to integrin heterodimers on the cell membrane via its disintegrin ligand/domain and suppresses cell migration by altering integrin-mediated signals. ADAM9-S might be involved in proteolyzing specific substrates on the cell surface or ECM that are not targeted by ADAM9-L. Subsequent degradation of the ECM caused by the ADAM9-S metalloprotease activity triggers cell migration and breast cancer metastasis

in tumor cells with their capacity to release tumor-promoting factors [216]. Several ADAMs have been analyzed in various tumors such as gastric, colon, breast, pancreatic, and renal cancers, ductal adenocarcinoma, prostatic adenocarcinoma, and skin melanoma where they are reported as differentially expressed and produce isoform-specific functions [216, 217].

2.4.1 ADAM9

ADAM9 (encoded by the gene *ADAM9*), one of the key members of the ADAM family, is reported to be overexpressed in several cancers and has the capacity to induce cell invasion and metastasis [98, 100, 214]. *ADAM9* overexpression modulates adhesion molecules and their sensitivity to growth factors, thus increasing cell adhesion and invasion in NSCLC [99]. Giebeler et al. showed that ADAM9 cleaves the laminin-β3 chain, modulates endothelial cell adhesion, and alters basement membrane integrity in melanoma metastasis [218]. Overexpression of *ADAM9* was shown to promote prostate epithelial hyperplasia and intraepithelial neoplasia in mice [219]. Furthermore, ADAM9 was found to be involved in the androgen-independent metastatic conversion of prostate cancer [216, 219]. *ADAM9* is alternatively spliced to produce membrane-bound and secreted protein isoforms ADAM9-L (length, 819 aa; mass, 90.5 kDa) and ADAM9-S (length, 655 aa; mass, 72.3 kDa) [98, 100, 220]. The shorter ADAM9-S isoform produced by the alternative splicing of exon 12 contains a unique 8 aa sequence compared with ADAM9-L and, in addition, inserts a premature stop codon [100]. The full-length transcript has an N-terminal signal peptide and a single hydrophobic region that codes for the transmembrane and cytoplasmic domains [100]. The cytoplasmic domain of ADAM9-L interacts with SH3 domain-containing proteins [220]. Soluble ADAM9-S lacks the transmembrane domain and cytoplasmic domain and is released from the cell via the secretory pathway [100, 220]. *ADAM9-S* is highly expressed in the liver and heart compared with other tissues [220]. ADAM9-S, secreted by hepatic cells, promotes invasion of hepatocellular carcinoma [100]. In colon cancer, ADAM9-S induces tumor invasion by its metalloprotease activities [100]. Indeed, ADAM9-S protease activity seems to be required for migration and invasion in several human tumor

cell lines [100]. Higher ADAM9-S protein expression compared with ADAM9-L protein was observed in breast cancer cell lines with ADAM9-S shown to increase cell migration while ADAM9-L suppressed cell migration (Fig. 4) [98, 100]. The suppression of cell migration via ADAM9-L requires integrin binding by its disintegrin domain. Therefore, ADAM9-L acts as a ligand for specific integrin heterodimers altering integrin-mediated signals, either by altered integrin binding or by cooperating with a parallel signaling pathway which interacts with integrin receptors (Fig. 4) [98]. ADAM9-S might be involved with the proteolysis of specific substrates on the cell surface or ECM that are not targeted by ADAM9-L as its localization is restricted by membrane tethering (Fig. 4). These results indicate the significance of *ADAM9* alternative splicing in cancer cell invasion and metastasis and suggest a possible use of ADAM9 isoforms in breast cancer prognosis.

2.4.2 ADAM12

ADAM12 (encoded by the gene *ADAM12*) is an another member of the ADAM family, which is lowly expressed in most normal human tissues, yet found highly expressed in tumors such as breast, liver, bladder, prostate, and lung tumors and glioblastomas [101, 105, 221–229]. Also, ADAM12 has been identified as a promoter of tumor cell invasion and metastasis [101, 102, 230]. Eckert et al. found that the knockdown of *ADAM12* considerably reduced the formation of invadopodia, degradation of ECM, and turnover of focal adhesion, and thereby increased cell adhesion in cancer cells [230]. Strikingly, soluble ADAM12 proteins have been suggested as diagnostic biomarkers in several cancers as well as an indicator of cancer progression [104, 222, 231]. Gilpin et al. demonstrated that alternative splicing of *ADAM12* produces two major protein isoforms ADAM12-L (length, 909 aa; mass, 99.5 kDa) and ADAM12-S (length, 738 aa; mass, 80.4 kDa) in humans (Table 1) [232]. The *ADAM12-L* splice variant contains exons 1 to 24 excluding exon 19, while the *ADAM12-S* variant contains only those exons up to exon 19 [103]. These two splice variants contain diverse 3′ UTRs, polyadenylation sites and altered carboxyl terminus in their respective isoforms [103, 232]. It has been found that these ADAM12 isoforms have distinct and opposing biological functions that have evolved due to their distinctive cellular localizations. ADAM12-L has a large cytoplasmic domain which is ideally positioned and localized to the cell membrane for its normal metalloprotease function [103]. ADAM12-L has been shown to shed epidermal growth factor receptor (EGFR) ligands, endothelial cell adhesion proteins, and endothelial receptor tyrosine kinase proteins [233–237]. The cytosolic domain of ADAM12-L interacts with the Src tyrosine kinase pathway to influence invadopodia formation in tumor cells, facilitating tumor cell invasion [221, 238]. This domain also binds with integrin-linked kinase (ILK) for integrin activation, in order to activate the pro-survival PI3K/Akt pathway [239]. Compared with ADAM12-L, ADAM12-S has a unique 34 aa carboxyl terminus, but no transmembrane and cytoplasmic domains, hence is secreted [232]. ADAM12-S was first identified in human placental cells and tumor cells such as lung adenocarcinoma and undifferentiated rhabdomyosarcoma [232]. Due to the altered orientation, ADAM12-S is unable to shed molecules from the cell membrane [232]. Although Roy et al. have shown that high amounts of ADAM12-S in urine in breast cancer correlates with more advanced disease, Nyren-Erickson et al. showed that urinary ADAM12-S levels did not vary considerably between pre-operative breast cancer patients and control participants [104, 240]. In breast cancer, ADAM12-S expression was found significantly associated with tumor invasion and metastasis, which can be related to its capability of degrading ECM components [104, 105]. Contradictorily, the overexpression of *ADAM12-L* variant was not found to be associated with breast cancer metastasis [104]. Muggy and colleagues reported that overexpression of *ADAM12-L* or *ADAM12-S* has no correlation with hormone receptor positive breast cancer prognosis, whereas *ADAM12-L* was found to be correlated with poor prognosis of triple negative breast cancers [103]. In addition, this study showed that ADAM12-L can enhance tumor growth by activating EGFR pathways [103].

2.4.3 ADAM15

ADAM15 (encoded by the gene *ADAM15*) was found to have an upregulated expression in a wide range of human tissues [241, 242]. In addition, elevated ADAM15 expression has been observed in hematologic malignancies, and in ovarian, gastric, breast, prostate, and lung cancers and brain tumors, possibly via its role in ectodomain shedding of cadherins [106–111]. Interactions of the disintegrin domain of ADAM15 with integrins have been reported in previous studies, which describe the mechanistic involvement of ADAM15-A, in regulating cell–cell adhesion and ECM modifications, promoting metastatic spread [243, 244]. Currently, 23 exons were identified in the *ADAM15* gene, which shows differential alternative exon use (exons 18–22) in human tissues [245]. A recent study by Zhong et al. has cloned and characterized four alternatively spliced naturally occurring ADAM15 isoforms, in human mammary carcinoma [112]. All four isoforms ADAM15-A to ADAM15-D were found derived by the alternative splicing of exons 18 to 23. The *ADAM15-A* variant codes for the full-length ADAM15 isoform (length, 814 aa; mass, 87.8 kDa). The shortest splice variant, *ADAM15-D*, is generated due to the exclusion of exons 19 to 21, causing a frame shift in exons 22 and 23, compared with other three isoforms (Table 1). Truncated ADAM15-D isoform lacks proline-rich modules and has a unique sequence of 37 aa prior to the premature stop codon.

Compared with *ADAM15-A*, *ADAM15-B* and *ADAM15-C* have 75-bp (20a) and 72-bp (20a+21) insertions between exons 19 and 22 (Table 1). However, all other isoforms were found with proline-rich motifs, with exons 19 and 20, introducing extra modules into the core cytoplasmic domain. Interestingly, the complex pattern of exon usage of *ADAM15* was found restricted to the cytoplasmic domain, whereas the extracellular region remained unchanged in all variants.

Zhong et al. have demonstrated that there were no significant changes in *ADAM15-A* and *ADAM15-D* transcript levels, yet *ADAM15-B* and *ADAM15-C* levels were significantly upregulated in breast cancer tissues compared with normal tissues [112]. This result is in accordance with the findings of Ortiz et al., who previously recorded no significant variation in total *ADAM15* transcript levels between normal mammary epithelial cells and breast cancer cell lines [109]. In addition, Zhong et al. observed that *ADAM15-A* overexpression does not alter cell morphology, but it can promote cell adhesion, migration, and invasion in MDA-MB-435 breast cancer cell lines [112]. Elevated *ADAM15-A* and *ADAM15-B* expression levels were found to be correlated with poorer survival in node-negative patients [112]. This suggests that these isoforms affect the growth of the primary tumor and promote dissemination of tumor cells at the primary site, but have less effect on nodal metastasis. Interestingly, *ADAM15-C* expression was correlated with enhanced survival in node-positive patients [112]. Curiously, more metastatic foci were observed with *ADAM15-B* expressing cells compared with *ADAM15-A* transfectants [112]. This was explained by the significant interaction of ADAM15-B proteins observed with extracellular signal-regulated kinase and the adaptor molecules [112]. The wide range of selectivity of ADAM15-B might have evolved due to their additional proline-rich motifs, which creates a binding platform to trigger cell signaling pathways that alter cell–cell adhesions and ECM arrangement, inducing cancer metastasis [112]. The cytoplasmic domains of ADAM15-A, ADAM15-B, and ADAM15-C have been reported with equal abilities to interact with these signaling and adaptor molecules. In summary, this study proposed the potential influence of ADAM15 isoforms on the tumor microenvironment, either at the site of tumor origin or in metastatic foci, suggesting their complex associations with mammary cancer development [112].

Transmembrane isoforms of ADAMs are involved in regulating cell signaling pathways via ectodomain shedding of cell membrane proteins. On the contrary, secretory isoforms of ADAMs were often found associated with disordered protein–protein interactions and altered cell signaling. The abovementioned studies have proposed a model in which ADAMs produced by both tumor and tumor-associated cells induce cancer metastasis through their interaction with various intra- or extracellular signaling and adaptor molecules. However, further studies are required to elucidate the differential functioning of ADAM isoforms in cancer metastasis *in vivo*.

3 Differential influence of therapeutic agents on alternative splicing of proteases

Many studies have highlighted the consequences of aberrant mRNA splice variants in triggering tumorigenesis and drug resistance, suggesting that alternative splicing is an intrinsic mechanism leading to therapy resistance [11]. Anti-cancer drugs may shift the alternative splicing of protease genes, leading to the dysregulation of protease isoform expression, conferring resistance to cancer therapy. A study carried out by Lai et al. has assessed whether alternative transcripts of *KLK* genes are differentially regulated by androgen-targeted therapy (use of anti-androgens to block the androgen-receptor signaling axis) as they can be used as indicators of therapy resistance to prostate cancer [120]. An overall decrease in expression of *KLK2* variants was found in LNCaP cells with the anti-androgens: bicalutamide and enzalutamide treatment, compared with the androgen: dihydrotestosterone (DHT) treatment [120]. Similarly, an overall decrease in expression of *KLK3* variants was detected with anti-androgen treatment although they showed lower responsiveness to the anti-androgen treatment compared with that of *KLK2* [120]. Michael et al. demonstrated the tissue-specific expression of *KLK4* splice variants in prostate cancer and their regulation by DHT and the androgenic progestin drug: norgestrel treatment [30]. Furthermore, the study revealed the downregulation of the *KLK4-IRIII* variant with the anti-inflammatory drug: dexamethasone treatment [30].

Since all caspase variants are involved in tumor growth and progression, use of caspase inhibitors have been noted for their promising role in cancer therapy [246]. Alternatively, using inducers of caspase expression and suppressing caspase inhibitor activities in carcinogenesis allows to maintain certain levels of proteolytic activities triggering tumor cell necrosis or apoptosis. As an example, the anti-cancerous and DNA-damaging agent etoposide (VP-16) is often used to induce caspase-2L-mediated cell apoptosis, conferring sensitization of tumor cells to chemotherapy [61]. Conflictingly, Wotawa et al. have demonstrated that VP-16 can also stimulate the production of the short *caspase-2s* anti-apoptotic splice variant in leukemic cells [247]. Although there is no effect detected on the 5′-end of the mRNA species, VP-16 significantly affects specifically the 3′-end of the *CASP2* gene [247]. Interestingly, relative production of the full-length *caspase-2L* splice variant together with pro-caspase-2L protein was significantly reduced compared with the short splice variant under VP-16 treatment. Similarly, another study also reported that both topoisomerase-I inhibitors (e.g., camptothecin and homocamptothecin derivatives) and topoisomerase-II

inhibitors (e.g., VP-16, amsacrine, doxorubicin, mitoxantrone) can induce the exon 9 retention of the *caspase-2s* splice variant in leukemic cells [248]. The study carried out by Vegran et al. has shown increased levels of caspase-3s/caspase-3L ratios in breast carcinomas with no pathological response to the neoadjuvant cyclophosphamide-containing chemotherapy used for the population examined [71]. The study showed that complete response to chemotherapy is dependent on the presence of the caspase-3L form yet needs a decrease in caspase-3s expression. As indicated by these studies, overexpression of an apoptotic inhibitor isoform and downregulation of the pro-apoptotic isoform, which resulted in an early cell response to cytotoxic agents, might confer resistance to the selected chemotherapy [62, 64, 249].

A differential effect of therapeutic agents on caspase 9 alternative splicing was also reported in previous studies. As an example, the chemotherapeutic agent, erlotinib, was found to increase the caspase-9a/9b ratio in NSCLC [88]. In addition, another DNA-damaging agent, cisplatin, was reported to trigger the apoptotic caspase-9a expression in PC3 prostate cancer cells [86]. On the other hand, a significant downregulation of the anti-apoptotic caspase-9b isoform was observed with the chemotherapeutic agent, gemcitabine, used in lung cancer treatment [250]. Furthermore, differential splicing patterns of *CASP9* in different cancer cell lines were observed with the anti-protozoal drug: emetine treatment [251].

Many of the above examples disclose that alternative splicing of proteases has become a legitimate mechanism of resistance to anti-cancer drugs/therapy, which is far more frequent than previously envisioned [11, 17]. One possible strategy to increase chemotherapeutic sensitivity of tumor cells is to regulate the alternative splicing of proteases critically associated with chemotherapeutic resistance. Targeting the core spliceosome machinery, targeting splicing regulators and use of oligonucleotide-based therapy are three of the main approaches currently used to modulate alternative splicing machinery [252]. In addition, characterization of tumor transcriptomes using high-throughput sequencing can be used to recognize splice variants associated with cancer-specific tumor characteristics.

4 Conclusion

In living systems, a balance between proteolytic and anti-proteolytic activities is maintained, and any disturbance to this balance can lead to various diseases, including cancer. Proteases have long been known as modulators of the tumor microenvironment, owing to their potential to affect cell signaling and modify/degrade ECM, which in turn accelerates cell migration and invasion towards cancer metastasis. Up to date, a growing body of studies showed evidence for the altered expression, altered activity, and altered localization of isoforms of all key protease classes in a wide range of cancers. Generation of protease isoforms with distinct and opposing roles in cancer can be attributed to the altered processing of protease splice variants in cancer cells. Therefore, characterization of the cancer degradome, with respect to the effects of alternative splicing, will become imperative to comprehend their effect on the different hallmarks of cancer progression. Continuous effort in understanding regulatory mechanisms involved in cancer-associated splicing events will aid in developing opposing and specific therapeutic strategies to halt cancer progression.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Lopez-Otin, C., & Overall, C. M. (2002). Protease degradomics: a new challenge for proteomics. *Nature Reviews. Molecular Cell Biology, 3*(7), 509–519. https://doi.org/10.1038/nrm858.
2. Puente, X. S., Ordóñez, G. R., & López-Otín, C. (2008). Protease genomics and the cancer degradome. In G. Høyer-Hansen, D. Edwards, F. Blasi, & B. F. Sloane (Eds.), *The cancer degradome* (pp. 3–15). New York: Springer.
3. Lopez-Otin, C., & Matrisian, L. M. (2007). Emerging roles of proteases in tumour suppression. *Nature Reviews. Cancer, 7*(10), 800–808. https://doi.org/10.1038/nrc2228.
4. Bond, J. S. (2019). Proteases: history, discovery, and roles in health and disease. *The Journal of Biological Chemistry, 294*(5), 1643–1651. https://doi.org/10.1074/jbc.TM118.004156.
5. Buo, L., Aasen, A. O., Karlsrud, T. S., Johansen, H. T., & Sivertsen, S. M. (1990). The role of proteases in the growth, invasion and spread of cancer cells. *Tidsskrift for den Norske Lægeforening, 110*(29), 3753–3756.
6. Duffy, M. J. (1992). The role of proteolytic enzymes in cancer invasion and metastasis. *Clinical & Experimental Metastasis, 10*(3), 145–155.
7. Friedl, P., & Wolf, K. (2008). Tube travel: the role of proteases in individual and collective cancer cell invasion. *Cancer Research, 68*(18), 7247–7249. https://doi.org/10.1158/0008-5472.CAN-08-0784.
8. Zucker, S. (1988). A critical appraisal of the role of proteolytic enzymes in cancer invasion: emphasis on tumor surface proteinases. *Cancer Investigation, 6*(2), 219–231.
9. Yang, Y., Hong, H., Zhang, Y., & Cai, W. (2009). Molecular imaging of proteases in cancer. *Cancer Growth Metastasis, 2*, 13–27.
10. El Marabti, E., & Younis, I. (2018). The cancer spliceome: reprograming of alternative splicing in cancer. *Frontiers in Molecular Biosciences, 5*, 80. https://doi.org/10.3389/fmolb.2018.00080.

11. Wang, B. D., & Lee, N. H. (2018). Aberrant RNA splicing in cancer and drug resistance. *Cancers (Basel), 10*(11). https://doi.org/10.3390/cancers10110458.
12. David, C. J., & Manley, J. L. (2010). Alternative pre-mRNA splicing regulation in cancer: pathways and programs unhinged. *Genes & Development, 24*(21), 2343–2364. https://doi.org/10.1101/gad.1973010.
13. Vegran, F., Boidot, R., Solary, E., & Lizard-Nacol, S. (2011). A short caspase-3 isoform inhibits chemotherapy-induced apoptosis by blocking apoptosome assembly. *PLoS One, 6*(12), e29058. https://doi.org/10.1371/journal.pone.0029058.
14. Brawerman, G. (1987). Determinants of messenger RNA stability. *Cell, 48*(1), 5–6. https://doi.org/10.1016/0092-8674(87)90346-1.
15. Young, R. A., Hagenbuchle, O., & Schibler, U. (1981). A single mouse alpha-amylase gene specifies two different tissue-specific mRNAs. *Cell, 23*(2), 451–458. https://doi.org/10.1016/0092-8674(81)90140-9.
16. Gong, Q., Chan, S. J., Bajkowski, A. S., Steiner, D. F., & Frankfater, A. (1993). Characterization of the cathepsin B gene and multiple mRNAs in human tissues: evidence for alternative splicing of cathepsin B pre-mRNA. *DNA and Cell Biology, 12*(4), 299–309. https://doi.org/10.1089/dna.1993.12.299.
17. Rehman, S. U., Husain, M. A., Sarwar, T., Ishqi, H. M., & Tabish, M. (2015). Modulation of alternative splicing by anticancer drugs. *Wiley Interdisciplinary Rev RNA, 6*(4), 369–379. https://doi.org/10.1002/wrna.1283.
18. Ishii, K., Otsuka, T., Iguchi, K., Usui, S., Yamamoto, H., Sugimura, Y., et al. (2004). Evidence that the prostate-specific antigen (PSA)/Zn2+ axis may play a role in human prostate cancer cell invasion. *Cancer Letters, 207*(1), 79–87. https://doi.org/10.1016/j.canlet.2003.09.029.
19. Webber, M. M., Waghray, A., & Bello, D. (1995). Prostate-specific antigen, a serine protease, facilitates human prostate cancer cell invasion. *Clinical Cancer Research, 1*(10), 1089–1094.
20. Fortier, A. H., Nelson, B. J., Grella, D. K., & Holaday, J. W. (1999). Antiangiogenic activity of prostate-specific antigen. *Journal of the National Cancer Institute, 91*(19), 1635–1640. https://doi.org/10.1093/jnci/91.19.1635.
21. Heidtmann, H. H., Nettelbeck, D. M., Mingels, A., Jager, R., Welker, H. G., & Kontermann, R. E. (1999). Generation of angiostatin-like fragments from plasminogen by prostate-specific antigen. *British Journal of Cancer, 81*(8), 1269–1273. https://doi.org/10.1038/sj.bjc.6692167.
22. Heuze-Vourc'h, N., Leblond, V., & Courty, Y. (2003). Complex alternative splicing of the hKLK3 gene coding for the tumor marker PSA (prostate-specific-antigen). *European Journal of Biochemistry, 270*(4), 706–714. https://doi.org/10.1046/j.1432-1033.2003.03425.x.
23. Heuze, N., Olayat, S., Gutman, N., Zani, M. L., & Courty, Y. (1999). Molecular cloning and expression of an alternative hKLK3 transcript coding for a variant protein of prostate-specific antigen. *Cancer Research, 59*(12), 2820–2824.
24. Whitbread, A. K., Veveris-Lowe, T. L., Dong, Y., Tan, O. L., Gardiner, R., Samaratunga, H. M., et al. (2010). Expression of PSA-RP2, an alternatively spliced variant from the PSA gene, is increased in prostate cancer tissues but the protein is not secreted from prostate cancer cells. *Biological Chemistry, 391*(4), 461–466. https://doi.org/10.1515/BC.2010.043.
25. Tanaka, T., Isono, T., Yoshiki, T., Yuasa, T., & Okada, Y. (2000). A novel form of prostate-specific antigen transcript produced by alternative splicing. *Cancer Research, 60*(1), 56–59.
26. Pampalakis, G., Scorilas, A., & Sotiropoulou, G. (2008). Novel splice variants of prostate-specific antigen and applications in diagnosis of prostate cancer. *Clinical Biochemistry, 41*(7-8), 591–597. https://doi.org/10.1016/j.clinbiochem.2007.12.022.
27. Borgono, C. A., & Diamandis, E. P. (2004). The emerging roles of human tissue kallikreins in cancer. *Nature Reviews. Cancer, 4*(11), 876–890. https://doi.org/10.1038/nrc1474.
28. Dong, Y., Bui, L. T., Odorico, D. M., Tan, O. L., Myers, S. A., Samaratunga, H., et al. (2005). Compartmentalized expression of kallikrein 4 (KLK4/hK4) isoforms in prostate cancer: nuclear, cytoplasmic and secreted forms. *Endocrine-Related Cancer, 12*(4), 875–889. https://doi.org/10.1677/erc.1.01062.
29. Myers, S. A., & Clements, J. A. (2001). Kallikrein 4 (KLK4), a new member of the human kallikrein gene family is up-regulated by estrogen and progesterone in the human endometrial cancer cell line, KLE. *The Journal of Clinical Endocrinology and Metabolism, 86*(5), 2323–2326. https://doi.org/10.1210/jcem.86.5.7625.
30. Michael, I. P., Kurlender, L., Memari, N., Yousef, G. M., Du, D., Grass, L., et al. (2005). Intron retention: a common splicing event within the human kallikrein gene family. *Clinical Chemistry, 51*(3), 506–515. https://doi.org/10.1373/clinchem.2004.042341.
31. Ogawa, K., Utsunomiya, T., Mimori, K., Tanaka, F., Inoue, H., Nagahara, H., et al. (2005). Clinical significance of human kallikrein gene 6 messenger RNA expression in colorectal cancer. *Clinical Cancer Research, 11*(8), 2889–2893. https://doi.org/10.1158/1078-0432.CCR-04-2281.
32. Klucky, B., Mueller, R., Vogt, I., Teurich, S., Hartenstein, B., Breuhahn, K., et al. (2007). Kallikrein 6 induces E-cadherin shedding and promotes cell proliferation, migration, and invasion. *Cancer Research, 67*(17), 8198–8206. https://doi.org/10.1158/0008-5472.CAN-07-0607.
33. Sananes, A., Cohen, I., Shahar, A., Hockla, A., De Vita, E., Miller, A. K., et al. (2018). A potent, proteolysis-resistant inhibitor of kallikrein-related peptidase 6 (KLK6) for cancer therapy, developed by combinatorial engineering. *The Journal of Biological Chemistry, 293*(33), 12663–12680. https://doi.org/10.1074/jbc.RA117.000871.
34. Pampalakis, G., Kurlender, L., Diamandis, E. P., & Sotiropoulou, G. (2004). Cloning and characterization of novel isoforms of the human kallikrein 6 gene. *Biochemical and Biophysical Research Communications, 320*(1), 54–61. https://doi.org/10.1016/j.bbrc.2004.04.205.
35. Sher, Y. P., Chou, C. C., Chou, R. H., Wu, H. M., Wayne Chang, W. S., Chen, C. H., et al. (2006). Human kallikrein 8 protease confers a favorable clinical outcome in non-small cell lung cancer by suppressing tumor cell invasiveness. *Cancer Research, 66*(24), 11763–11770. https://doi.org/10.1158/0008-5472.CAN-06-3165.
36. Magklara, A., Scorilas, A., Katsaros, D., Massobrio, M., Yousef, G. M., Fracchioli, S., et al. (2001). The human KLK8 (neuropsin/ovasin) gene: identification of two novel splice variants and its prognostic value in ovarian cancer. *Clinical Cancer Research, 7*(4), 806–811.
37. Planque, C., Choi, Y. H., Guyetant, S., Heuze-Vourc'h, N., Briollais, L., & Courty, Y. (2010). Alternative splicing variant of kallikrein-related peptidase 8 as an independent predictor of unfavorable prognosis in lung cancer. *Clinical Chemistry, 56*(6), 987–997. https://doi.org/10.1373/clinchem.2009.138917.
38. Bengsch, F., Buck, A., Gunther, S. C., Seiz, J. R., Tacke, M., Pfeifer, D., et al. (2014). Cell type-dependent pathogenic functions of overexpressed human cathepsin B in murine breast cancer progression. *Oncogene, 33*(36), 4474–4484. https://doi.org/10.1038/onc.2013.395.
39. Chen, Q., Fei, J., Wu, L., Jiang, Z., Wu, Y., Zheng, Y., et al. (2011). Detection of cathepsin B, cathepsin L, cystatin C, urokinase plasminogen activator and urokinase plasminogen activator receptor in the sera of lung cancer patients. *Oncology Letters, 2*(4), 693–699. https://doi.org/10.3892/ol.2011.302.
40. Fujise, N., Nanashim, A., Taniguchi, Y., Matsuo, S., Hatano, K., Matsumoto, Y., et al. (2000). Prognostic impact of cathepsin B and

matrix metalloproteinase-9 in pulmonary adenocarcinomas by immunohistochemical study. *Lung Cancer, 27*(1), 19–26.
41. Krueger, S., Haeckel, C., Buehling, F., & Roessner, A. (1999). Inhibitory effects of antisense cathepsin B cDNA transfection on invasion and motility in a human osteosarcoma cell line. *Cancer Research, 59*(23), 6010–6014.
42. Rempel, S. A., Rosenblum, M. L., Mikkelsen, T., Yan, P. S., Ellis, K. D., Golembieski, W. A., et al. (1994). Cathepsin B expression and localization in glioma progression and invasion. *Cancer Research, 54*(23), 6027–6031.
43. Sevenich, L., Werner, F., Gajda, M., Schurigt, U., Sieber, C., Muller, S., et al. (2011). Transgenic expression of human cathepsin B promotes progression and metastasis of polyoma-middle-T-induced breast cancer in mice. *Oncogene, 30*(1), 54–64. https://doi.org/10.1038/onc.2010.387.
44. Sloane, B. F., Dunn, J. R., & Honn, K. V. (1981). Lysosomal cathepsin B: correlation with metastatic potential. *Science, 212*(4499), 1151–1153. https://doi.org/10.1126/science.7233209.
45. Vasiljeva, O., Korovin, M., Gajda, M., Brodoefel, H., Bojic, L., Kruger, A., et al. (2008). Reduced tumour cell proliferation and delayed development of high-grade mammary carcinomas in cathepsin B-deficient mice. *Oncogene, 27*(30), 4191–4199. https://doi.org/10.1038/onc.2008.59.
46. Vasiljeva, O., Papazoglou, A., Kruger, A., Brodoefel, H., Korovin, M., Deussing, J., et al. (2006). Tumor cell-derived and macrophage-derived cathepsin B promotes progression and lung metastasis of mammary cancer. *Cancer Research, 66*(10), 5242–5250. https://doi.org/10.1158/0008-5472.CAN-05-4463.
47. Withana, N. P., Blum, G., Sameni, M., Slaney, C., Anbalagan, A., Olive, M. B., et al. (2012). Cathepsin B inhibition limits bone metastasis in breast cancer. *Cancer Research, 72*(5), 1199–1209. https://doi.org/10.1158/0008-5472.CAN-11-2759.
48. Wu, D., Wang, H. J., Li, Z. N., Wang, L. H., Zheng, F. Y., Jiang, J., et al. (2012). Cathepsin B may be a potential biomarker in cervical cancer. *Histology and Histopathology, 27*(1), 79–87.
49. Mehtani, S., Gong, Q., Panella, J., Subbiah, S., Peffley, D. M., & Frankfater, A. (1998). In vivo expression of an alternatively spliced human tumor message that encodes a truncated form of cathepsin B. Subcellular distribution of the truncated enzyme in COS cells. *The Journal of Biological Chemistry, 273*(21), 13236–13244. https://doi.org/10.1074/jbc.273.21.13236.
50. Tholen, M., Wolanski, J., Stolze, B., Chiabudini, M., Gajda, M., Bronsert, P., et al. (2015). Stress-resistant translation of cathepsin L mRNA in breast cancer progression. *The Journal of Biological Chemistry, 290*(25), 15758–15769. https://doi.org/10.1074/jbc.M114.624353.
51. Rescheleit, D. K., Rommerskirch, W. J., & Wiederanders, B. (1996). Sequence analysis and distribution of two new human cathepsin L splice variants. *FEBS Letters, 394*(3), 345–348.
52. Arora, S., & Chauhan, S. S. (2002). Identification and characterization of a novel human cathepsin L splice variant. *Gene, 293*(1-2), 123–131. https://doi.org/10.1016/s0378-1119(02)00700-x.
53. Abudula, A., Rommerskirch, W., Weber, E., Gunther, D., & Wiederanders, B. (2001). Splice variants of human cathepsin L mRNA show different expression rates. *Biological Chemistry, 382*(11), 1583–1591. https://doi.org/10.1515/BC.2001.193.
54. Goulet, B., Sansregret, L., Leduy, L., Bogyo, M., Weber, E., Chauhan, S. S., et al. (2007). Increased expression and activity of nuclear cathepsin L in cancer cells suggests a novel mechanism of cell transformation. *Molecular Cancer Research, 5*(9), 899–907. https://doi.org/10.1158/1541-7786.MCR-07-0160.
55. Chauhan, S. S., Popescu, N. C., Ray, D., Fleischmann, R., Gottesman, M. M., & Troen, B. R. (1993). Cloning, genomic organization, and chromosomal localization of human cathepsin L. *The Journal of Biological Chemistry, 268*(2), 1039–1045.
56. Estrov, Z., Thall, P. F., Talpaz, M., Estey, E. H., Kantarjian, H. M., Andreeff, M., et al. (1998). Caspase 2 and caspase 3 protein levels as predictors of survival in acute myelogenous leukemia. *Blood, 92*(9), 3090–3097.
57. Faderl, S., Thall, P. F., Kantarjian, H. M., Talpaz, M., Harris, D., Van, Q., et al. (1999). Caspase 2 and caspase 3 as predictors of complete remission and survival in adults with acute lymphoblastic leukemia. *Clinical Cancer Research, 5*(12), 4041–4047.
58. Kim, M. S., Kim, H. S., Jeong, E. G., Soung, Y. H., Yoo, N. J., & Lee, S. H. (2011). Somatic mutations of caspase-2 gene in gastric and colorectal cancers. *Pathology, Research and Practice, 207*(10), 640–644. https://doi.org/10.1016/j.prp.2011.08.004.
59. Kumar, S., White, D. L., Takai, S., Turczynowicz, S., Juttner, C. A., & Hughes, T. P. (1995). Apoptosis regulatory gene NEDD2 maps to human chromosome segment 7q34-35, a region frequently affected in haematological neoplasms. *Human Genetics, 95*(6), 641–644. https://doi.org/10.1007/bf00209480.
60. Ren, K., Lu, J., Porollo, A., & Du, C. (2012). Tumor-suppressing function of caspase-2 requires catalytic site Cys-320 and site Ser-139 in mice. *The Journal of Biological Chemistry, 287*(18), 14792–14802. https://doi.org/10.1074/jbc.M112.347625.
61. Droin, N., Dubrez, L., Eymin, B., Renvoize, C., Breard, J., Dimanche-Boitrel, M. T., et al. (1998). Upregulation of CASP genes in human tumor cells undergoing etoposide-induced apoptosis. *Oncogene, 16*(22), 2885–2894. https://doi.org/10.1038/sj.onc.1201821.
62. Droin, N., Beauchemin, M., Solary, E., & Bertrand, R. (2000). Identification of a caspase-2 isoform that behaves as an endogenous inhibitor of the caspase cascade. *Cancer Research, 60*(24), 7039–7047.
63. Han, C., Zhao, R., Kroger, J., Qu, M., Wani, A. A., & Wang, Q. E. (2013). Caspase-2 short isoform interacts with membrane-associated cytoskeleton proteins to inhibit apoptosis. *PLoS One, 8*(7), e67033. https://doi.org/10.1371/journal.pone.0067033.
64. Parent, N., Sane, A. T., Droin, N., & Bertrand, R. (2005). Procaspase-2S inhibits procaspase-3 processing and activation, preventing ROCK-1-mediated apoptotic blebbing and body formation in human B lymphoma Namalwa cells. *Apoptosis, 10*(2), 313–322. https://doi.org/10.1007/s10495-005-0805-7.
65. Toh, W. H., Logette, E., Corcos, L., & Sabapathy, K. (2008). TAp73 beta and DNp73 beta activate the expression of the pro-survival caspase-2(S). *Nucleic Acids Research, 36*(13), 4498–4509. https://doi.org/10.1093/nar/gkn414.
66. Huang, S. C., Tang, M. J., Hsu, K. F., Cheng, Y. M., & Chou, C. Y. (2002). Fas and its ligand, caspases, and bcl-2 expression in gonadotropin-releasing hormone agonist-treated uterine leiomyoma. *The Journal of Clinical Endocrinology and Metabolism, 87*(10), 4580–4586. https://doi.org/10.1210/jc.2001-011968.
67. Huang, Y., Shin, N. H., Sun, Y., & Wang, K. K. (2001). Molecular cloning and characterization of a novel caspase-3 variant that attenuates apoptosis induced by proteasome inhibition. *Biochemical and Biophysical Research Communications, 283*(4), 762–769. https://doi.org/10.1006/bbrc.2001.4871.
68. Liu, Y. R., Sun, B., Zhao, X. L., Gu, Q., Liu, Z. Y., Dong, X. Y., et al. (2013). Basal caspase-3 activity promotes migration, invasion, and vasculogenic mimicry formation of melanoma cells. *Melanoma Research, 23*(4), 243–253. https://doi.org/10.1097/CMR.0b013e3283625498.
69. Mukai, M., Kusama, T., Hamanaka, Y., Koga, T., Endo, H., Tatsuta, M., et al. (2005). Cross talk between apoptosis and invasion signaling in cancer cells through caspase-3 activation. *Cancer Research, 65*(20), 9121–9125. https://doi.org/10.1158/0008-5472.Can-04-4344.

70. O'Donovan, N., Crown, J., Stunell, H., Hill, A. D., McDermott, E., O'Higgins, N., et al. (2003). Caspase 3 in breast cancer. *Clinical Cancer Research, 9*(2), 738–742.
71. Vegran, F., Boidot, R., Oudin, C., Riedinger, J. M., Bonnetain, F., & Lizard-Nacol, S. (2006). Overexpression of caspase-3s splice variant in locally advanced breast carcinoma is associated with poor response to neoadjuvant chemotherapy. *Clinical Cancer Research, 12*(19), 5794–5800. https://doi.org/10.1158/1078-0432.CCR-06-0725.
72. Woenckhaus, C., Giebel, J., Failing, K., Fenic, I., Dittberner, T., & Poetsch, M. (2003). Expression of AP-2alpha, c-kit, and cleaved caspase-6 and -3 in naevi and malignant melanomas of the skin. A possible role for caspases in melanoma progression? *The Journal of Pathology, 201*(2), 278–287. https://doi.org/10.1002/path.1424.
73. Zhao, X., Wang, D., Zhao, Z., Xiao, Y., Sengupta, S., Xiao, Y., et al. (2006). Caspase-3-dependent activation of calcium-independent phospholipase A2 enhances cell migration in non-apoptotic ovarian cancer cells. *The Journal of Biological Chemistry, 281*(39), 29357–29368. https://doi.org/10.1074/jbc.M513105200.
74. Barbero, S., Barila, D., Mielgo, A., Stagni, V., Clair, K., & Stupack, D. (2008). Identification of a critical tyrosine residue in caspase 8 that promotes cell migration. *The Journal of Biological Chemistry, 283*(19), 13031–13034. https://doi.org/10.1074/jbc.M800549200.
75. Barbero, S., Mielgo, A., Torres, V., Teitz, T., Shields, D. J., Mikolon, D., et al. (2009). Caspase-8 association with the focal adhesion complex promotes tumor cell migration and metastasis. *Cancer Research, 69*(9), 3755–3763. https://doi.org/10.1158/0008-5472.CAN-08-3937.
76. Helfer, B., Boswell, B. C., Finlay, D., Cipres, A., Vuori, K., Bong Kang, T., et al. (2006). Caspase-8 promotes cell motility and calpain activity under nonapoptotic conditions. *Cancer Research, 66*(8), 4273–4278. https://doi.org/10.1158/0008-5472.CAN-05-4183.
77. Senft, J., Helfer, B., & Frisch, S. M. (2007). Caspase-8 interacts with the p85 subunit of phosphatidylinositol 3-kinase to regulate cell adhesion and motility. *Cancer Research, 67*(24), 11505–11509. https://doi.org/10.1158/0008-5472.CAN-07-5755.
78. Teitz, T., Stupack, D. G., & Lahti, J. M. (2006). Halting neuroblastoma metastasis by controlling integrin-mediated death. *Cell Cycle, 5*(7), 681–685. https://doi.org/10.4161/cc.5.7.2615.
79. Miller, M. A., Karacay, B., Zhu, X., O'Dorisio, M. S., & Sandler, A. D. (2006). Caspase 8L, a novel inhibitory isoform of caspase 8, is associated with undifferentiated neuroblastoma. *Apoptosis, 11*(1), 15–24. https://doi.org/10.1007/s10495-005-3258-0.
80. Mohr, A., Zwacka, R. M., Jarmy, G., Buneker, C., Schrezenmeier, H., Dohner, K., et al. (2005). Caspase-8L expression protects CD34+ hematopoietic progenitor cells and leukemic cells from CD95-mediated apoptosis. *Oncogene, 24*(14), 2421–2429. https://doi.org/10.1038/sj.onc.1208432.
81. Finlay, D., Howes, A., & Vuori, K. (2009). Caspase-8 as a potential mediator of pro-tumorigenic signals. *Cell Cycle, 8*(21), 3441–3442. https://doi.org/10.4161/cc.8.21.9649.
82. Finlay, D., & Vuori, K. (2007). Novel noncatalytic role for caspase-8 in promoting Src-mediated adhesion and Erk signaling in neuroblastoma cells. *Cancer Research, 67*(24), 11704–11711. https://doi.org/10.1158/0008-5472.Can-07-1906.
83. Xu, Z., Tang, K., Wang, M., Rao, Q., Liu, B., & Wang, J. (2009). A new caspase-8 isoform caspase-8s increased sensitivity to apoptosis in Jurkat cells. *Journal of Biomedicine & Biotechnology, 2009*, 930462. https://doi.org/10.1155/2009/930462.
84. Chalfant, C. E., Rathman, K., Pinkerman, R. L., Wood, R. E., Obeid, L. M., Ogretmen, B., et al. (2002). De novo ceramide regulates the alternative splicing of caspase 9 and Bcl-x in A549 lung adenocarcinoma cells. Dependence on protein phosphatase-1. *The Journal of Biological Chemistry, 277*(15), 12587–12595. https://doi.org/10.1074/jbc.M112010200.
85. Gangwar, R., Mandhani, A., & Mittal, R. D. (2009). Caspase 9 and caspase 8 gene polymorphisms and susceptibility to bladder cancer in north Indian population. *Annals of Surgical Oncology, 16*(7), 2028–2034. https://doi.org/10.1245/s10434-009-0488-3.
86. Hagen, R. M., Chedea, V. S., Mintoff, C. P., Bowler, E., Morse, H. R., & Ladomery, M. R. (2013). Epigallocatechin-3-gallate promotes apoptosis and expression of the caspase 9a splice variant in PC3 prostate cancer cells. *International Journal of Oncology, 43*(1), 194–200. https://doi.org/10.3892/ijo.2013.1920.
87. Shultz, J. C., Goehe, R. W., Murudkar, C. S., Wijesinghe, D. S., Mayton, E. K., Massiello, A., et al. (2011). SRSF1 regulates the alternative splicing of caspase 9 via a novel intronic splicing enhancer affecting the chemotherapeutic sensitivity of non-small cell lung cancer cells. *Molecular Cancer Research, 9*(7), 889–900. https://doi.org/10.1158/1541-7786.MCR-11-0061.
88. Shultz, J. C., Goehe, R. W., Wijesinghe, D. S., Murudkar, C., Hawkins, A. J., Shay, J. W., et al. (2010). Alternative splicing of caspase 9 is modulated by the phosphoinositide 3-kinase/Akt pathway via phosphorylation of SRp30a. *Cancer Research, 70*(22), 9185–9196. https://doi.org/10.1158/0008-5472.Can-10-1545.
89. Theodoropoulos, G. E., Michalopoulos, N. V., Panoussopoulos, S. G., Taka, S., & Gazouli, M. (2010). Effects of caspase-9 and survivin gene polymorphisms in pancreatic cancer risk and tumor characteristics. *Pancreas, 39*(7), 976–980. https://doi.org/10.1097/MPA.0b013e3181d705d4.
90. Zhang, D., Liu, H., Yang, B., Hu, J., & Cheng, Y. (2019). L-securinine inhibits cell growth and metastasis of human androgen-independent prostate cancer DU145 cells via regulating mitochondrial and AGTR1/MEK/ERK/STAT3/PAX2 apoptotic pathways. *Bioscience Reports, 39*(5). https://doi.org/10.1042/BSR20190469.
91. Zhang, Y., Hou, Q., Li, X., Zhu, J., Wang, W., Li, B., et al. (2019). Enrichment of novel quinazoline derivatives with high antitumor activity in mitochondria tracked by its self-fluorescence. *European Journal of Medicinal Chemistry, 178*, 417–432. https://doi.org/10.1016/j.ejmech.2019.06.015.
92. Goehe, R. W., Shultz, J. C., Murudkar, C., Usanovic, S., Lamour, N. F., Massey, D. H., et al. (2010). hnRNP L regulates the tumorigenic capacity of lung cancer xenografts in mice via caspase-9 pre-mRNA processing. *The Journal of Clinical Investigation, 120*(11), 3923–3939. https://doi.org/10.1172/JCI43552.
93. Seol, D. W., & Billiar, T. R. (1999). A caspase-9 variant missing the catalytic site is an endogenous inhibitor of apoptosis. *Journal of Biological Chemistry, 274*(4), 2072–2076. https://doi.org/10.1074/jbc.274.4.2072.
94. Muhlethaler-Mottet, A., Flahaut, M., Bourloud, K. B., Nardou, K., Coulon, A., Liberman, J., et al. (2011). Individual caspase-10 isoforms play distinct and opposing roles in the initiation of death receptor-mediated tumour cell apoptosis. *Cell Death & Disease, 2*, e125. https://doi.org/10.1038/cddis.2011.8.
95. Engels, I. H., Totzke, G., Fischer, U., Schulze-Osthoff, K., & Janicke, R. U. (2005). Caspase-10 sensitizes breast carcinoma cells to TRAIL-induced but not tumor necrosis factor-induced apoptosis in a caspase-3-dependent manner. *Molecular and Cellular Biology, 25*(7), 2808–2818. https://doi.org/10.1128/MCB.25.7.2808-2818.2005.
96. Ng, P. W., Porter, A. G., & Janicke, R. U. (1999). Molecular cloning and characterization of two novel pro-apoptotic isoforms of caspase-10. *The Journal of Biological Chemistry, 274*(15), 10301–10308. https://doi.org/10.1074/jbc.274.15.10301.
97. Wang, H., Wang, P., Sun, X., Luo, Y., Wang, X., Ma, D., et al. (2007). Cloning and characterization of a novel caspase-10 isoform that activates NF-kappa B activity.

Biochimica et Biophysica Acta, 1770(11), 1528–1537. https://doi.org/10.1016/j.bbagen.2007.07.010.

98. Fry, J. L., & Toker, A. (2010). Secreted and membrane-bound isoforms of protease ADAM9 have opposing effects on breast cancer cell migration. *Cancer Research, 70*(20), 8187–8198. https://doi.org/10.1158/0008-5472.CAN-09-4231.
99. Shintani, Y., Higashiyama, S., Ohta, M., Hirabayashi, H., Yamamoto, S., Yoshimasu, T., et al. (2004). Overexpression of ADAM9 in non-small cell lung cancer correlates with brain metastasis. *Cancer Research, 64*(12), 4190–4196. https://doi.org/10.1158/0008-5472.CAN-03-3235.
100. Mazzocca, A., Coppari, R., De Franco, R., Cho, J. Y., Libermann, T. A., Pinzani, M., et al. (2005). A secreted form of ADAM9 promotes carcinoma invasion through tumor-stromal interactions. *Cancer Research, 65*(11), 4728–4738. https://doi.org/10.1158/0008-5472.CAN-04-4449.
101. Kveiborg, M., Frohlich, C., Albrechtsen, R., Tischler, V., Dietrich, N., Holck, P., et al. (2005). A role for ADAM12 in breast tumor progression and stromal cell apoptosis. *Cancer Research, 65*(11), 4754–4761. https://doi.org/10.1158/0008-5472.Can-05-0262.
102. Shao, S. H., Li, Z. L., Gao, W., Yu, G. H., Liu, D. X., & Pan, F. (2014). ADAM-12 as a diagnostic marker for the proliferation, migration and invasion in patients with small cell lung cancer. *PLoS One, 9*(1), e85936. https://doi.org/10.1371/journal.pone.0085936.
103. Duhachek Muggy, S. (2014). Multiple isoforms of ADAM12 in breast cancer: differential regulation of expression and unique roles in cancer progression. Kansas State University, K-State Electronic Theses, Dissertations, and Reports: 2004
104. Roy, R., Wewer, U. M., Zurakowski, D., Pories, S. E., & Moses, M. A. (2004). ADAM 12 cleaves extracellular matrix proteins and correlates with cancer status and stage. *The Journal of Biological Chemistry, 279*(49), 51323–51330. https://doi.org/10.1074/jbc.M409565200.
105. Roy, R., Rodig, S., Bielenberg, D., Zurakowski, D., & Moses, M. A. (2011). ADAM12 transmembrane and secreted isoforms promote breast tumor growth: a distinct role for ADAM12-S protein in tumor metastasis. *The Journal of Biological Chemistry, 286*(23), 20758–20768. https://doi.org/10.1074/jbc.M110.216036.
106. Carl-McGrath, S., Lendeckel, U., Ebert, M., Roessner, A., & Rocken, C. (2005). The disintegrin-metalloproteinases ADAM9, ADAM12, and ADAM15 are upregulated in gastric cancer. *International Journal of Oncology, 26*(1), 17–24.
107. Horiuchi, K., Weskamp, G., Lum, L., Hammes, H. P., Cai, H., Brodie, T. A., et al. (2003). Potential role for ADAM15 in pathological neovascularization in mice. *Molecular and Cellular Biology, 23*(16), 5614–5624. https://doi.org/10.1128/mcb.23.16.5614-5624.2003.
108. Kuefer, R., Day, K. C., Kleer, C. G., Sabel, M. S., Hofer, M. D., Varambally, S., et al. (2006). ADAM15 disintegrin is associated with aggressive prostate and breast cancer disease. *Neoplasia, 8*(4), 319–329. https://doi.org/10.1593/neo.05682.
109. Ortiz, R. M., Karkkainen, I., & Huovila, A. P. J. (2004). Aberrant alternative exon use and increased copy number of human metalloprotease-disintegrin ADAM15 gene in breast cancer cells. *Genes, Chromosomes & Cancer, 41*(4), 366–378. https://doi.org/10.1002/gcc.20102.
110. Schutz, A., Hartig, W., Wobus, M., Grosche, J., Wittekind, C., & Aust, G. (2005). Expression of ADAM15 in lung carcinomas. *Virchows Archiv, 446*(4), 421–429. https://doi.org/10.1007/s00428-004-1193-z.
111. Wu, E., Croucher, P. I., & McKie, N. (1997). Expression of members of the novel membrane linked metalloproteinase family ADAM in cells derived from a range of haematological malignancies. *Biochemical and Biophysical Research Communications, 235*(2), 437–442. https://doi.org/10.1006/bbrc.1997.6714.
112. Zhong, J. L., Poghosyan, Z., Pennington, C. J., Scott, X., Handsley, M. M., Warn, A., et al. (2008). Distinct functions of natural ADAM-15 cytoplasmic domain variants in human mammary carcinoma. *Molecular Cancer Research, 6*(3), 383–394. https://doi.org/10.1158/1541-7786.MCR-07-2028.
113. Hedstrom, L. (2002). Serine protease mechanism and specificity. *Chemical Reviews, 102*(12), 4501–4524.
114. Adamopoulos, P. G., Kontos, C. K., & Scorilas, A. (2018). Discovery of novel transcripts of the human tissue kallikrein (KLK1) and kallikrein-related peptidase 2 (KLK2) in human cancer cells, exploiting next-generation sequencing technology. *Genomics*. https://doi.org/10.1016/j.ygeno.2018.03.022.
115. Borgono, C. A., Michael, I. P., & Diamandis, E. P. (2004). Human tissue kallikreins: physiologic roles and applications in cancer. *Molecular Cancer Research, 2*(5), 257–280.
116. Wolf, W. C., Evans, D. M., Chao, L., & Chao, J. (2001). A synthetic tissue kallikrein inhibitor suppresses cancer cell invasiveness. *The American Journal of Pathology, 159*(5), 1797–1805. https://doi.org/10.1016/S0002-9440(10)63026-X.
117. Lai, J., An, J., Srinivasan, S., Clements, J. A., & Batra, J. (2016). A computational analysis of the genetic and transcript diversity at the kallikrein locus. *Biological Chemistry, 397*(12), 1307–1313. https://doi.org/10.1515/hsz-2016-0161.
118. Hong, S. K. (2014). Kallikreins as biomarkers for prostate cancer. *BioMed Research International, 2014*, 526341. https://doi.org/10.1155/2014/526341.
119. Balk, S. P., Ko, Y. J., & Bubley, G. J. (2003). Biology of prostate-specific antigen. *Journal of Clinical Oncology, 21*(2), 383–391. https://doi.org/10.1200/JCO.2003.02.083.
120. Lai, J., An, J., Nelson, C. C., Lehman, M. L., Batra, J., & Clements, J. A. (2014). Analysis of androgen and anti-androgen regulation of KLK-related peptidase 2, 3, and 4 alternative transcripts in prostate cancer. *Biological Chemistry, 395*(9), 1127–1132. https://doi.org/10.1515/hsz-2014-0149.
121. Riegman, P. H., Klaassen, P., van der Korput, J. A., Romijn, J. C., & Trapman, J. (1988). Molecular cloning and characterization of novel prostate antigen cDNA's. *Biochemical and Biophysical Research Communications, 155*(1), 181–188. https://doi.org/10.1016/s0006-291x(88)81066-0.
122. Gilgunn, S., Conroy, P. J., Saldova, R., Rudd, P. M., & O'Kennedy, R. J. (2013). Aberrant PSA glycosylation-a sweet predictor of prostate cancer. *Nature Reviews Urology, 10*(2), 99–107. https://doi.org/10.1038/nruro1.2012.258.
123. Obiezu, C. V., Soosaipillai, A., Jung, K., Stephan, C., Scorilas, A., Howarth, D. H., et al. (2002). Detection of human kallikrein 4 in healthy and cancerous prostatic tissues by immunofluorometry and immunohistochemistry. *Clinical Chemistry, 48*(8), 1232–1240.
124. Xi, Z. J., Klokk, T. I., Korkmaz, K., Kurys, P., Elbi, C., Risberg, B., et al. (2004). Kallikrein 4 is a predominantly nuclear protein and is overexpressed in prostate cancer. *Cancer Research, 64*(7), 2365–2370. https://doi.org/10.1158/0008-5472.Can-03-2025.
125. Kurlender, L., Borgono, C., Michael, I. P., Obiezu, C., Elliott, M. B., Yousef, G. M., et al. (2005). A survey of alternative transcripts of human tissue kallikrein genes. *Biochimica et Biophysica Acta, 1755*(1), 1–14. https://doi.org/10.1016/j.bbcan.2005.02.001.
126. Obiezu, C. V., & Diamandis, E. P. (2000). An alternatively spliced variant of KLK4 expressed in prostatic tissue. *Clinical Biochemistry, 33*(7), 599–600.
127. Dong, Y., Kaushal, A., Bui, L., Chu, S., Fuller, P. J., Nicklin, J., et al. (2001). Human kallikrein 4 (KLK4) is highly expressed in serous ovarian carcinomas. *Clinical Cancer Research, 7*(8), 2363–2371.

128. Korkmaz, K. S., Korkmaz, C. G., Pretlow, T. G., & Saatcioglu, F. (2001). Distinctly different gene structure of KLK4/KLK-L1/prostase/ARM1 compared with other members of the kallikrein family: intracellular localization, alternative cDNA forms, and Regulation by multiple hormones. *DNA and Cell Biology, 20*(7), 435–445. https://doi.org/10.1089/104454901750361497.
129. Klokk, T. I., Kilander, A., Xi, Z., Waehre, H., Risberg, B., Danielsen, H. E., et al. (2007). Kallikrein 4 is a proliferative factor that is overexpressed in prostate cancer. *Cancer Research, 67*(11), 5221–5230. https://doi.org/10.1158/0008-5472.CAN-06-4728.
130. Veveris-Lowe, T. L., Lawrence, M. G., Collard, R. L., Bui, L., Herington, A. C., Nicol, D. L., et al. (2005). Kallikrein 4 (hK4) and prostate-specific antigen (PSA) are associated with the loss of E-cadherin and an epithelial-mesenchymal transition (EMT)-like effect in prostate cancer cells. *Endocrine-Related Cancer, 12*(3), 631–643. https://doi.org/10.1677/erc.1.00958.
131. Wang, W., Mize, G. J., Zhang, X., & Takayama, T. K. (2010). Kallikrein-related peptidase-4 initiates tumor-stroma interactions in prostate cancer through protease-activated receptor-1. *International Journal of Cancer, 126*(3), 599–610. https://doi.org/10.1002/ijc.24904.
132. Pampalakis, G., Prosnikli, E., Agalioti, T., Vlahou, A., Zoumpourlis, V., & Sotiropoulou, G. (2009). A tumor-protective role for human kallikrein-related peptidase 6 in breast cancer mediated by inhibition of epithelial-to-mesenchymal transition. *Cancer Research, 69*(9), 3779–3787. https://doi.org/10.1158/0008-5472.CAN-08-1976.
133. Kuzmanov, U., Jiang, N., Smith, C. R., Soosaipillai, A., & Diamandis, E. P. (2009). Differential N-glycosylation of kallikrein 6 derived from ovarian cancer cells or the central nervous system. *Molecular & Cellular Proteomics, 8*(4), 791–798. https://doi.org/10.1074/mcp.M800516-MCP200.
134. Nagahara, H., Mimori, K., Utsunomiya, T., Barnard, G. F., Ohira, M., Hirakawa, K., et al. (2005). Clinicopathologic and biological significance of kallikrein 6 overexpression in human gastric cancer. *Clinical Cancer Research, 11*(19 Pt 1), 6800–6806. https://doi.org/10.1158/1078-0432.CCR-05-0943.
135. Adamopoulos, P. G., Kontos, C. K., & Scorilas, A. (2017). Molecular cloning of novel transcripts of human kallikrein-related peptidases 5, 6, 7, 8 and 9 (KLK5 - KLK9), using Next-generation sequencing. *Scientific Reports, 7*(1), 17299. https://doi.org/10.1038/s41598-017-16269-6.
136. Bayani, J., & Diamandis, E. P. (2011). The physiology and pathobiology of human kallikrein-related peptidase 6 (KLK6). *Clinical Chemistry and Laboratory Medicine, 50*(2), 211–233. https://doi.org/10.1515/CCLM.2011.750.
137. Cane, S., Bignotti, E., Bellone, S., Palmieri, M., De las Casas, L., Roman, J. J., et al. (2004). The novel serine protease tumor-associated differentially expressed gene-14 (KLK8/Neuropsin/Ovasin) is highly overexpressed in cervical cancer. *American Journal of Obstetrics and Gynecology, 190*(1), 60–66. https://doi.org/10.1016/j.ajog.2003.07.020.
138. Darling, M. R., Tsai, S., Jackson-Boeters, L., Daley, T. D., & Diamandis, E. P. (2008). Human kallikrein 8 expression in salivary gland tumors. *Head and Neck Pathology, 2*(3), 169–174. https://doi.org/10.1007/s12105-008-0068-z.
139. Jin, H., Nagai, N., Shigemasa, K., Gu, L., Tanimoto, H., Yunokawa, M., et al. (2006). Expression of tumor-associated differentially expressed gene-14 (TADG-14/KLK8) and its protein hK8 in uterine endometria and endometrial carcinomas. *Tumour Biology, 27*(5), 274–282. https://doi.org/10.1159/000094741.
140. Liu, X., Quan, B., Tian, Z., Xi, H., Jia, G., Wang, H., et al. (2017). Elevated expression of KLK8 predicts poor prognosis in colorectal cancer. *Biomedicine & Pharmacotherapy, 88*, 595–602. https://doi.org/10.1016/j.biopha.2017.01.112.
141. Shigemasa, K., Tian, X., Gu, L., Tanimoto, H., Underwood, L. J., O'Brien, T. J., et al. (2004). Human kallikrein 8 (hK8/TADG-14) expression is associated with an early clinical stage and favorable prognosis in ovarian cancer. *Oncology Reports, 11*(6), 1153–1159.
142. Liu, C. J., Liu, T. Y., Kuo, L. T., Cheng, H. W., Chu, T. H., Chang, K. W., et al. (2008). Differential gene expression signature between primary and metastatic head and neck squamous cell carcinoma. *The Journal of Pathology, 214*(4), 489–497. https://doi.org/10.1002/path.2306.
143. Lu, Z. X., Huang, Q., & Su, B. (2009). Functional characterization of the human-specific (type II) form of kallikrein 8, a gene involved in learning and memory. *Cell Research, 19*(2), 259–267. https://doi.org/10.1038/cr.2009.4.
144. Mohamed, M. M., & Sloane, B. F. (2006). Cysteine cathepsins: multifunctional enzymes in cancer. *Nature Reviews. Cancer, 6*(10), 764–775. https://doi.org/10.1038/nrc1949.
145. Tan, G. J., Peng, Z. K., Lu, J. P., & Tang, F. Q. (2013). Cathepsins mediate tumor metastasis. *World Journal of Biological Chemistry, 4*(4), 91–101. https://doi.org/10.4331/wjbc.v4.i4.91.
146. Turk, V., Stoka, V., Vasiljeva, O., Renko, M., Sun, T., Turk, B., et al. (2012). Cysteine cathepsins: from structure, function and regulation to new frontiers. *Biochimica et Biophysica Acta, 1824*(1), 68–88. https://doi.org/10.1016/j.bbapap.2011.10.002.
147. Aggarwal, N., & Sloane, B. F. (2014). Cathepsin B: multiple roles in cancer. *Proteomics. Clinical Applications, 8*(5-6), 427–437. https://doi.org/10.1002/prca.201300105.
148. Kusunoki, T., Nishida, S., Nakano, T., Funasaka, K., Kimoto, S., Murata, K., et al. (1995). Study on cathepsin B activity in human thyroid tumors. *Auris Nasus Larynx, 22*(1), 43–48.
149. Victor, B. C., Anbalagan, A., Mohamed, M. M., Sloane, B. F., & Cavallo-Medved, D. (2011). Inhibition of cathepsin B activity attenuates extracellular matrix degradation and inflammatory breast cancer invasion. *Breast Cancer Research, 13*(6), R115. https://doi.org/10.1186/bcr3058.
150. Yan, S., Sameni, M., & Sloane, B. F. (1998). Cathepsin B and human tumor progression. *Biological Chemistry, 379*(2), 113–123.
151. Vidak, E., Javorsek, U., Vizovisek, M., & Turk, B. (2019). Cysteine cathepsins and their extracellular roles: shaping the microenvironment. *Cells, 8*(3), 264. https://doi.org/10.3390/cells8030264.
152. Berquin, I. M., Ahram, M., & Sloane, B. F. (1997). Exon 2 of human cathepsin B derives from an Alu element. *FEBS Letters, 419*(1), 121–123.
153. Baici, A., Muntener, K., Willimann, A., & Zwicky, R. (2006). Regulation of human cathepsin B by alternative mRNA splicing: homeostasis, fatal errors and cell death. *Biological Chemistry, 387*(8), 1017–1021. https://doi.org/10.1515/BC.2006.125.
154. Muntener, K., Zwicky, R., Csucs, G., Rohrer, J., & Baici, A. (2004). Exon skipping of cathepsin B: mitochondrial targeting of a lysosomal peptidase provokes cell death. *The Journal of Biological Chemistry, 279*(39), 41012–41017. https://doi.org/10.1074/jbc.M405333200.
155. Lowy, D. R., & Willumsen, B. M. (1993). Function and regulation of ras. *Annual Review of Biochemistry, 62*, 851–891. https://doi.org/10.1146/annurev.bi.62.070193.004223.
156. Quilliam, L. A., Khosravi-Far, R., Huff, S. Y., & Der, C. J. (1995). Guanine nucleotide exchange factors: activators of the Ras superfamily of proteins. *Bioessays, 17*(5), 395–404. https://doi.org/10.1002/bies.950170507.
157. Senda, T., Matsuno, K., & Mita, S. (1997). The presence of sigma receptor subtypes in bovine retinal membranes. *Experimental Eye Research, 64*(5), 857–860. https://doi.org/10.1006/exer.1996.0272.
158. Muntener, K., Willimann, A., Zwicky, R., Svoboda, B., Mach, L., & Baici, A. (2005). Folding competence of N-

terminally truncated forms of human procathepsin B. *The Journal of Biological Chemistry, 280*(12), 11973–11980. https://doi.org/10.1074/jbc.M413052200.
159. Bestvater, F., Dallner, C., & Spiess, E. (2005). The C-terminal subunit of artificially truncated human cathepsin B mediates its nuclear targeting and contributes to cell viability. *BMC Cell Biology, 6*(1), 16. https://doi.org/10.1186/1471-2121-6-16.
160. Chauhan, S. S., Goldstein, L. J., & Gottesman, M. M. (1991). Expression of cathepsin L in human tumors. *Cancer Research, 51*(5), 1478–1481.
161. Sudhan, D. R., & Siemann, D. W. (2015). Cathepsin L targeting in cancer treatment. *Pharmacology & Therapeutics, 155*, 105–116. https://doi.org/10.1016/j.pharmthera.2015.08.007.
162. Zhang, L. S., Wei, L. X., Shen, G. Z., He, B. F., Gong, W., Min, N., et al. (2015). Cathepsin L is involved in proliferation and invasion of ovarian cancer cells. *Molecular Medicine Reports, 11*(1), 468–474. https://doi.org/10.3892/mmr.2014.2706.
163. Colella, R., & Casey, S. F. (2003). Decreased activity of cathepsins L + B and decreased invasive ability of PC3 prostate cancer cells. *Biotechnic & Histochemistry, 78*(2), 101–108.
164. Laurent-Matha, V., Derocq, D., Prebois, C., Katunuma, N., & Liaudet-Coopman, E. (2006). Processing of human cathepsin D is independent of its catalytic function and auto-activation: involvement of cathepsins L and B. *Journal of Biochemistry, 139*(3), 363–371. https://doi.org/10.1093/jb.mvj037.
165. Caserman, S., Kenig, S., Sloane, B. F., & Lah, T. T. (2006). Cathepsin L splice variants in human breast cell lines. *Biological Chemistry, 387*(5), 629–634. https://doi.org/10.1515/BC.2006.080.
166. Mittal, S., Mir, R. A., & Chauhan, S. S. (2011). Post-transcriptional regulation of human cathepsin L expression. *Biological Chemistry, 392*(5), 405–413. https://doi.org/10.1515/BC.2011.039.
167. Godet, A. C., David, F., Hantelys, F., Tatin, F., Lacazette, E., Garmy-Susini, B., et al. (2019). IRES trans-acting factors, key actors of the stress response. *International Journal of Molecular Sciences, 20*(4). https://doi.org/10.3390/ijms20040924.
168. Jean, D., Rousselet, N., & Frade, R. (2008). Cathepsin L expression is up-regulated by hypoxia in human melanoma cells: role of its 5'-untranslated region. *Biochemical Journal, 413*, 125–134. https://doi.org/10.1042/Bj20071255.
169. Zhong, Y. J., Shao, L. H., & Li, Y. (2013). Cathepsin B-cleavable doxorubicin prodrugs for targeted cancer therapy (Review). *International Journal of Oncology, 42*(2), 373–383. https://doi.org/10.3892/ijo.2012.1754.
170. McIlwain, D. R., Berger, T., & Mak, T. W. (2015). Caspase functions in cell death and disease. *Cold Spring Harbor Perspectives in Biology, 7*(4). https://doi.org/10.1101/cshperspect.a026716.
171. Chang, H. Y., & Yang, X. (2000). Proteases for cell suicide: functions and regulation of caspases. *Microbiology and Molecular Biology Reviews, 64*(4), 821–846. https://doi.org/10.1128/mmbr.64.4.821-846.2000.
172. Kumar, S. (2007). Caspase function in programmed cell death. *Cell Death and Differentiation, 14*(1), 32–43. https://doi.org/10.1038/sj.cdd.4402060.
173. Wyllie, A. H. (1997). Apoptosis and carcinogenesis. *European Journal of Cell Biology, 73*(3), 189–197.
174. Chen, Q., Jin, M., Yang, F., Zhu, J., Xiao, Q., & Zhang, L. (2013). Matrix metalloproteinases: inflammatory regulators of cell behaviors in vascular formation and remodeling. *Mediators of Inflammation, 2013*, 928315. https://doi.org/10.1155/2013/928315.
175. Suzanne, M., & Steller, H. (2009). Letting go: modification of cell adhesion during apoptosis. *Journal of Biology, 8*(5), 49. https://doi.org/10.1186/jbiol152.
176. Xu, D. C., Arthurton, L., & Baena-Lopez, L. A. (2018). Learning on the fly: the interplay between caspases and cancer. *BioMed Research International, 2018*, 5473180. https://doi.org/10.1155/2018/5473180.
177. Nicholson, D. W. (1999). Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death and Differentiation, 6*(11), 1028–1042. https://doi.org/10.1038/sj.cdd.4400598.
178. Horiuchi, T., Himeji, D., Tsukamoto, H., Harashima, S., Hashimura, C., & Hayashi, K. (2000). Dominant expression of a novel splice variant of caspase-8 in human peripheral blood lymphocytes. *Biochemical and Biophysical Research Communications, 272*(3), 877–881. https://doi.org/10.1006/bbrc.2000.2841.
179. Schwerk, C., & Schulze-Osthoff, K. (2005). Regulation of apoptosis by alternative pre-mRNA splicing. *Molecular Cell, 19*(1), 1–13. https://doi.org/10.1016/j.molcel.2005.05.026.
180. Jelinek, M., Balusikova, K., Kopperova, D., Nemcova-Furstova, V., Sramek, J., Fidlerova, J., et al. (2013). Caspase-2 is involved in cell death induction by taxanes in breast cancer cells. *Cancer Cell International, 13*, 42. https://doi.org/10.1186/1475-2867-13-42.
181. Kumar, S., Kinoshita, M., & Noda, M. (1997). Characterization of a mammalian cell death gene Nedd2. *Leukemia, 11*(*Suppl 3*), 385–386.
182. Wang, L., Miura, M., Bergeron, L., Zhu, H., & Yuan, J. (1994). Ich-1, an Ice/ced-3-related gene, encodes both positive and negative regulators of programmed cell death. *Cell, 78*(5), 739–750. https://doi.org/10.1016/s0092-8674(94)90422-7.
183. Fushimi, K., Ray, P., Kar, A., Wang, L., Sutherland, L. C., & Wu, J. Y. (2008). Up-regulation of the proapoptotic caspase 2 splicing isoform by a candidate tumor suppressor, RBM5. *Proceedings of the National Academy of Sciences of the United States of America, 105*(41), 15708–15713. https://doi.org/10.1073/pnas.0805569105.
184. Droin, N., Rebe, C., Bichat, F., Hammann, A., Bertrand, R., & Solary, E. (2001). Modulation of apoptosis by procaspase-2 short isoform: selective inhibition of chromatin condensation, apoptotic body formation and phosphatidylserine externalization. *Oncogene, 20*(2), 260–269. https://doi.org/10.1038/sj.onc.1204066.
185. Ito, A., Uehara, T., & Nomura, Y. (2000). Isolation of Ich-1S (caspase-2S)-binding protein that partially inhibits caspase activity. *FEBS Letters, 470*(3), 360–364. https://doi.org/10.1016/s0014-5793(00)01351-x.
186. Logette, E., Wotawa, A., Solier, S., Desoche, L., Solary, E., & Corcos, L. (2003). The human caspase-2 gene: alternative promoters, pre-mRNA splicing and AUG usage direct isoform-specific expression. *Oncogene, 22*(6), 935–946. https://doi.org/10.1038/sj.onc.1206172.
187. Solier, S., Logette, E., Desoche, L., Solary, E., & Corcos, L. (2005). Nonsense-mediated mRNA decay among human caspases: the caspase-2S putative protein is encoded by an extremely short-lived mRNA. *Cell Death and Differentiation, 12*(6), 687–689. https://doi.org/10.1038/sj.cdd.4401594.
188. Baker, K. E., & Parker, R. (2004). Nonsense-mediated mRNA decay: terminating erroneous gene expression. *Current Opinion in Cell Biology, 16*(3), 293–299. https://doi.org/10.1016/j.ceb.2004.03.003.
189. Rohn, T. T., Cusack, S. M., Kessinger, S. R., & Oxford, J. T. (2004). Caspase activation independent of cell death is required for proper cell dispersal and correct morphology in PC12 cells. *Experimental Cell Research, 295*(1), 215–225. https://doi.org/10.1016/j.yexcr.2003.12.029.
190. Brentnall, M., Weir, D. B., Rongvaux, A., Marcus, A. I., & Boise, L. H. (2014). Procaspase-3 regulates fibronectin secretion and influences adhesion, migration and survival independently of

catalytic function. *Journal of Cell Science, 127*(Pt 10), 2217–2226. https://doi.org/10.1242/jcs.135137.

191. Gdynia, G., Grund, K., Eckert, A., Bock, B. C., Funke, B., Macher-Goeppinger, S., et al. (2007). Basal caspase activity promotes migration and invasiveness in glioblastoma cells. *Molecular Cancer Research, 5*(12), 1232–1240. https://doi.org/10.1158/1541-7786.MCR-07-0343.
192. Grenet, J., Teitz, T., Wei, T., Valentine, V., & Kidd, V. J. (1999). Structure and chromosome localization of the human CASP8 gene. *Gene, 226*(2), 225–232. https://doi.org/10.1016/s0378-1119(98)00565-4.
193. Graf, R. P., Keller, N., Barbero, S., & Stupack, D. (2014). Caspase-8 as a regulator of tumor cell motility. *Current Molecular Medicine, 14*(2), 246–254. https://doi.org/10.2174/1566524014666140128111951.
194. Keller, N., Ozmadenci, D., Ichim, G., & Stupack, D. (2018). Caspase-8 function, and phosphorylation, in cell migration. *Seminars in Cell & Developmental Biology, 82*, 105–117. https://doi.org/10.1016/j.semcdb.2018.01.009.
195. Stupack, D. G., Teitz, T., Potter, M. D., Mikolon, D., Houghton, P. J., Kidd, V. J., et al. (2006). Potentiation of neuroblastoma metastasis by loss of caspase-8. *Nature, 439*(7072), 95–99. https://doi.org/10.1038/nature04323.
196. Raguenez, G., Muhlethaler-Mottet, A., Meier, R., Duros, C., Benard, J., & Gross, N. (2009). Fenretinide-induced caspase-8 activation and apoptosis in an established model of metastatic neuroblastoma. *BMC Cancer, 9*, 97. https://doi.org/10.1186/1471-2407-9-97.
197. Scaffidi, C., Medema, J. P., Krammer, P. H., & Peter, M. E. (1997). FLICE is predominantly expressed as two functionally active isoforms, caspase-8/a and caspase-8/b. *Journal of Biological Chemistry, 272*(43), 26953–26958. https://doi.org/10.1074/jbc.272.43.26953.
198. Fernandes-Alnemri, T., Armstrong, R. C., Krebs, J., Srinivasula, S. M., Wang, L., Bullrich, F., et al. (1996). In vitro activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains. *Proceedings of the National Academy of Sciences of the United States of America, 93*(15), 7464–7469. https://doi.org/10.1073/pnas.93.15.7464.
199. Finlay, D., Howes, A., & Vuori, K. (2009). Critical Role for Caspase-8 in epidermal growth factor signaling. *Cancer Research, 69*(12), 5023–5029. https://doi.org/10.1158/0008-5472.Can-08-3731.
200. Kim, B., Srivastava, S. K., & Kim, S. H. (2015). Caspase-9 as a therapeutic target for treating cancer. *Expert Opinion on Therapeutic Targets, 19*(1), 113–127. https://doi.org/10.1517/14728222.2014.961425.
201. Soung, Y. H., Lee, J. W., Kim, S. Y., Park, W. S., Nam, S. W., Lee, J. Y., et al. (2006). Mutational analysis of proapoptotic caspase-9 gene in common human carcinomas. *APMIS, 114*(4), 292–297. https://doi.org/10.1111/j.1600-0463.2006.apm_364.x.
202. Ekert, P. G., Read, S. H., Silke, J., Marsden, V. S., Kaufmann, H., Hawkins, C. J., et al. (2004). Apaf-1 and caspase-9 accelerate apoptosis, but do not determine whether factor-deprived or drug-treated cells die. *The Journal of Cell Biology, 165*(6), 835–842. https://doi.org/10.1083/jcb.200312031.
203. Marsden, V. S., O'Connor, L., O'Reilly, L. A., Silke, J., Metcalf, D., Ekert, P. G., et al. (2002). Apoptosis initiated by Bcl-2-regulated caspase activation independently of the cytochrome c/Apaf-1/caspase-9 apoptosome. *Nature, 419*(6907), 634–637. https://doi.org/10.1038/nature01101.
204. Li, P., Zhou, L., Zhao, T., Liu, X., Zhang, P., Liu, Y., et al. (2017). Caspase-9: structure, mechanisms and clinical application. *Oncotarget, 8*(14), 23996–24008. https://doi.org/10.18632/oncotarget.15098.
205. Srinivasula, S. M., Ahmad, M., Guo, Y., Zhan, Y., Lazebnik, Y., Fernandes-Alnemri, T., et al. (1999). Identification of an endogenous dominant-negative short isoform of caspase-9 that can regulate apoptosis. *Cancer Research, 59*(5), 999–1002.
206. Vu, N. T., Park, M. A., Shultz, J. C., Goehe, R. W., Hoeferlin, L. A., Shultz, M. D., et al. (2013). hnRNP U enhances caspase-9 splicing and is modulated by AKT-dependent phosphorylation of hnRNP L. *The Journal of Biological Chemistry, 288*(12), 8575–8584. https://doi.org/10.1074/jbc.M112.443333.
207. Kischkel, F. C., Lawrence, D. A., Tinel, A., LeBlanc, H., Virmani, A., Schow, P., et al. (2001). Death receptor recruitment of endogenous caspase-10 and apoptosis initiation in the absence of caspase-8. *The Journal of Biological Chemistry, 276*(49), 46639–46646. https://doi.org/10.1074/jbc.M105102200.
208. Harada, K., Toyooka, S., Shivapurkar, N., Maitra, A., Reddy, J. L., Matta, H., et al. (2002). Deregulation of caspase 8 and 10 expression in pediatric tumors and cell lines. *Cancer Research, 62*(20), 5897–5901.
209. Park, W. S., Lee, J. H., Shin, M. S., Park, J. Y., Kim, H. S., Lee, J. H., et al. (2002). Inactivating mutations of the caspase-10 gene in gastric cancer. *Oncogene, 21*(18), 2919–2925. https://doi.org/10.1038/sj.onc.1205394.
210. Shin, M. S., Kim, H. S., Kang, C. S., Park, W. S., Kim, S. Y., Lee, S. N., et al. (2002). Inactivating mutations of CASP10 gene in non-Hodgkin lymphomas. *Blood, 99*(11), 4094–4099. https://doi.org/10.1182/blood.v99.11.4094.
211. Shin, M. S., Kim, H. S., Lee, S. H., Lee, J. W., Song, Y. H., Kim, Y. S., et al. (2002). Alterations of Fas-pathway genes associated with nodal metastasis in non-small cell lung cancer. *Oncogene, 21*(26), 4129–4136. https://doi.org/10.1038/sj.onc.1205527.
212. Horn, S., Hughes, M. A., Schilling, R., Sticht, C., Tenev, T., Ploesser, M., et al. (2017). Caspase-10 Negatively regulates caspase-8-mediated cell death, switching the response to CD95L in favor of NF-kappaB activation and cell survival. *Cell Reports, 19*(4), 785–797. https://doi.org/10.1016/j.celrep.2017.04.010.
213. Olsson, M., & Zhivotovsky, B. (2011). Caspases and cancer. *Cell Death and Differentiation, 18*(9), 1441–1449. https://doi.org/10.1038/cdd.2011.30.
214. Peduto, L. (2009). ADAM9 as a potential target molecule in cancer. *Current Pharmaceutical Design, 15*(20), 2282–2287. https://doi.org/10.2174/138161209788682415.
215. Stone, A. L., Kroeger, M., & Sang, Q. X. (1999). Structure-function analysis of the ADAM family of disintegrin-like and metalloproteinase-containing proteins (review). *Journal of Protein Chemistry, 18*(4), 447–465.
216. Duffy, M. J., McKiernan, E., O'Donovan, N., & McGowan, P. M. (2009). Role of ADAMs in cancer formation and progression. *Clinical Cancer Research, 15*(4), 1140–1144. https://doi.org/10.1158/1078-0432.CCR-08-1585.
217. Fritzsche, F. R., Wassermann, K., Jung, M., Tolle, A., Kristiansen, I., Lein, M., et al. (2008). ADAM9 is highly expressed in renal cell cancer and is associated with tumour progression. *BMC Cancer, 8*, 179. https://doi.org/10.1186/1471-2407-8-179.
218. Giebeler, N., Schonefuss, A., Landsberg, J., Tuting, T., Mauch, C., & Zigrino, P. (2017). Deletion of ADAM-9 in HGF/CDK4 mice impairs melanoma development and metastasis. *Oncogene, 36*(35), 5058–5067. https://doi.org/10.1038/onc.2017.162.
219. Peduto, L., Reuter, V. E., Shaffer, D. R., Scher, H. I., & Blobel, C. P. (2005). Critical function for ADAM9 in mouse prostate cancer. *Cancer Research, 65*(20), 9312–9319. https://doi.org/10.1158/0008-5472.Can-05-1063.
220. Hotoda, N., Koike, H., Sasagawa, N., & Ishiura, S. (2002). A secreted form of human ADAM9 has an alpha-secretase activity for APP. *Biochemical and Biophysical Research Communications, 293*(2), 800–805. https://doi.org/10.1016/S0006-291X(02)00302-9.

221. Albrechtsen, R., Stautz, D., Sanjay, A., Kveiborg, M., & Wewer, U. M. (2011). Extracellular engagement of ADAM12 induces clusters of invadopodia with localized ectodomain shedding activity. *Experimental Cell Research, 317*(2), 195–209. https://doi.org/10.1016/j.yexcr.2010.10.003.
222. Frohlich, C., Albrechtsen, R., Dyrskjot, L., Rudkjaer, L., Orntoft, T. F., & Wewer, U. M. (2006). Molecular profiling of ADAM12 in human bladder cancer. *Clinical Cancer Research, 12*(24), 7359–7368. https://doi.org/10.1158/1078-0432.CCR-06-1066.
223. Iba, K., Albrechtsen, R., Gilpin, B. J., Loechel, F., & Wewer, U. M. (1999). Cysteine-rich domain of human ADAM 12 (meltrin alpha) supports tumor cell adhesion. *The American Journal of Pathology, 154*(5), 1489–1501. https://doi.org/10.1016/s0002-9440(10)65403-x.
224. Kodama, T., Ikeda, E., Okada, A., Ohtsuka, T., Shimoda, M., Shiomi, T., et al. (2004). ADAM12 is selectively overexpressed in human glioblastomas and is associated with glioblastoma cell proliferation and shedding of heparin-binding epidermal growth factor. *American Journal of Pathology, 165*(5), 1743–1753. https://doi.org/10.1016/S0002-9440(10)63429-3.
225. Lendeckel, U., Kohl, J., Arndt, M., Carl-McGrath, S., Donat, H., & Rocken, C. (2005). Increased expression of ADAM family members in human breast cancer and breast cancer cell lines. *Journal of Cancer Research and Clinical Oncology, 131*(1), 41–48. https://doi.org/10.1007/s00432-004-0619-y.
226. Mino, N., Miyahara, R., Nakayama, E., Takahashi, T., Takahashi, A., Iwakiri, S., et al. (2009). A disintegrin and metalloprotease 12 (ADAM12) is a prognostic factor in resected pathological stage I lung adenocarcinoma. *Journal of Surgical Oncology, 100*(3), 267–272. https://doi.org/10.1002/jso.21313.
227. Narita, D., Anghel, A., Seclaman, E., Ilina, R., Cireap, N., & Ursoniu, S. (2010). Molecular profiling of ADAM12 gene in breast cancers. *Romanian Journal of Morphology and Embryology, 51*(4), 669–676.
228. Peduto, L., Reuter, V. E., Sehara-Fujisawa, A., Shaffer, D. R., Scher, H. I., & Blobel, C. P. (2006). ADAM12 is highly expressed in carcinoma-associated stroma and is required for mouse prostate tumor progression. *Oncogene, 25*(39), 5462–5466. https://doi.org/10.1038/sj.onc.1209536.
229. Rocks, N., Paulissen, G., Quesada Calvo, F., Polette, M., Gueders, M., Munaut, C., et al. (2006). Expression of a disintegrin and metalloprotease (ADAM and ADAMTS) enzymes in human non-small-cell lung carcinomas (NSCLC). *British Journal of Cancer, 94*(5), 724–730. https://doi.org/10.1038/sj.bjc.6602990.
230. Eckert, M. A., Santiago-Medina, M., Lwin, T. M., Kim, J., Courtneidge, S. A., & Yang, J. (2017). ADAM12 induction by Twist1 promotes tumor invasion and metastasis via regulation of invadopodia and focal adhesions. *Journal of Cell Science, 130*(12), 2036–2048. https://doi.org/10.1242/jcs.198200.
231. Pories, S. E., Zurakowski, D., Roy, R., Lamb, C. C., Raza, S., Exarhopoulos, A., et al. (2008). Urinary metalloproteinases: non-invasive biomarkers for breast cancer risk assessment. *Cancer Epidemiology, Biomarkers & Prevention, 17*(5), 1034–1042. https://doi.org/10.1158/1055-9965.EPI-07-0365.
232. Gilpin, B. J., Loechel, F., Mattei, M. G., Engvall, E., Albrechtsen, R., & Wewer, U. M. (1998). A novel, secreted form of human ADAM 12 (meltrin alpha) provokes myogenesis in vivo. *The Journal of Biological Chemistry, 273*(1), 157–166. https://doi.org/10.1074/jbc.273.1.157.
233. Diaz, B., Yuen, A., Iizuka, S., Higashiyama, S., & Courtneidge, S. A. (2013). Notch increases the shedding of HB-EGF by ADAM12 to potentiate invadopodia formation in hypoxia. *The Journal of Cell Biology, 201*(2), 279–292. https://doi.org/10.1083/jcb.201209151.
234. Dyczynska, E., Sun, D., Yi, H., Sehara-Fujisawa, A., Blobel, C. P., & Zolkiewska, A. (2007). Proteolytic processing of delta-like 1 by ADAM proteases. *The Journal of Biological Chemistry, 282*(1), 436–444. https://doi.org/10.1074/jbc.M605451200.
235. Frohlich, C., Klitgaard, M., Noer, J. B., Kotzsch, A., Nehammer, C., Kronqvist, P., et al. (2013). ADAM12 is expressed in the tumour vasculature and mediates ectodomain shedding of several membrane-anchored endothelial proteins. *Biochemical Journal, 452*, 97–109. https://doi.org/10.1042/Bj20121558.
236. Horiuchi, K., Le Gall, S., Schulte, M., Yamaguchi, T., Reiss, K., Murphy, G., et al. (2007). Substrate selectivity of epidermal growth factor-receptor ligand sheddases and their regulation by phorbol esters and calcium influx. *Molecular Biology of the Cell, 18*(1), 176–188. https://doi.org/10.1091/mbc.e06-01-0014.
237. Ohlig, S., Farshi, P., Pickhinke, U., van den Boom, J., Hoing, S., Jakuschev, S., et al. (2011). Sonic hedgehog shedding results in functional activation of the solubilized protein. *Developmental Cell, 20*(6), 764–774. https://doi.org/10.1016/j.devcel.2011.05.010.
238. Kang, Q., Cao, Y., & Zolkiewska, A. (2000). Metalloprotease-disintegrin ADAM 12 binds to the SH3 domain of Src and activates Src tyrosine kinase in C2C12 cells. *Biochemical Journal, 352*(*Pt 3*), 883–892.
239. Leyme, A., Bourd-Boittin, K., Bonnier, D., Falconer, A., Arlot-Bonnemains, Y., & Theret, N. (2012). Identification of ILK as a new partner of the ADAM12 disintegrin and metalloprotease in cell adhesion and survival. *Molecular Biology of the Cell, 23*(17), 3461–3472. https://doi.org/10.1091/mbc.E11-11-0918.
240. Nyren-Erickson, E. K., Bouton, M., Raval, M., Totzauer, J., Mallik, S., & Alberto, N. (2014). Urinary concentrations of ADAM 12 from breast cancer patients pre- and post-surgery vs. cancer-free controls: a clinical study for biomarker validation. *Journal of Negative Results in Biomedicine, 13*, 5. https://doi.org/10.1186/1477-5751-13-5.
241. Kratzschmar, J., Lum, L., & Blobel, C. P. (1996). Metargidin, a membrane-anchored metalloprotease-disintegrin protein with an RGD integrin binding sequence. *The Journal of Biological Chemistry, 271*(9), 4593–4596. https://doi.org/10.1074/jbc.271.9.4593.
242. Beck, V., Herold, H., Benge, A., Luber, B., Hutzler, P., Tschesche, H., et al. (2005). ADAM15 decreases integrin alphavbeta3/vitronectin-mediated ovarian cancer cell adhesion and motility in an RGD-dependent fashion. *The International Journal of Biochemistry & Cell Biology, 37*(3), 590–603. https://doi.org/10.1016/j.biocel.2004.08.005.
243. Nath, D., Slocombe, P. M., Stephens, P. E., Warn, A., Hutchinson, G. R., Yamada, K. M., et al. (1999). Interaction of metargidin (ADAM-15) with alphavbeta3 and alpha5beta1 integrins on different haemopoietic cells. *Journal of Cell Science, 112*(*Pt 4*), 579–587.
244. Eto, K., Puzon-McLaughlin, W., Sheppard, D., Sehara-Fujisawa, A., Zhang, X. P., & Takada, Y. (2000). RGD-independent binding of integrin alpha9beta1 to the ADAM-12 and -15 disintegrin domains mediates cell-cell interaction. *The Journal of Biological Chemistry, 275*(45), 34922–34930. https://doi.org/10.1074/jbc.M001953200.
245. Kleino, I., Ortiz, R. M., & Huovila, A. P. J. (2007). ADAM15 gene structure and differential alternative exon use in human tissues. *BMC Molecular Biology, 8*, 90. https://doi.org/10.1186/1471-2199-8-90.
246. Eatemadi, A., Aiyelabegan, H. T., Negahdari, B., Mazlomi, M. A., Daraee, H., Daraee, N., et al. (2017). Role of protease and protease inhibitors in cancer pathogenesis and treatment. *Biomedicine & Pharmacotherapy, 86*, 221–231. https://doi.org/10.1016/j.biopha.2016.12.021.
247. Wotawa, A., Solier, S., Logette, E., Solary, E., & Corcos, L. (2002). Differential influence of etoposide on two caspase-2

mRNA isoforms in leukemic cells. *Cancer Letters, 185*(2), 181–189.

248. Solier, S., Lansiaux, A., Logette, E., Wu, J., Soret, J., Tazi, J., et al. (2004). Topoisomerase I and II inhibitors control caspase-2 pre-messenger RNA splicing in human cells. *Molecular Cancer Research, 2*(1), 53–61.
249. Martinet, W., Knaapen, M. W., De Meyer, G. R., Herman, A. G., & Kockx, M. M. (2003). Overexpression of the anti-apoptotic caspase-2 short isoform in macrophage-derived foam cells of human atherosclerotic plaques. *The American Journal of Pathology, 162*(3), 731–736. https://doi.org/10.1016/S0002-9440(10)63869-2.
250. Patwardhan, G. A., & Liu, Y. Y. (2011). Sphingolipids and expression regulation of genes in cancer. *Progress in Lipid Research, 50*(1), 104–114. https://doi.org/10.1016/j.plipres.2010.10.003.
251. Pan, D., Boon-Unge, K., Govitrapong, P., & Zhou, J. (2011). Emetine regulates the alternative splicing of caspase 9 in tumor cells. *Oncology Letters, 2*(6), 1309–1312. https://doi.org/10.3892/ol.2011.395.
252. Lin, J. C. (2017). Therapeutic applications of targeted alternative splicing to cancer treatment. *International Journal of Molecular Sciences, 19*(1). https://doi.org/10.3390/ijms19010075.