Histol Histopathol (2006) 21: 1339-1353

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Ectopeptidases in tumour biology: A review

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Summary. Cell membrane-bound proteolytic enzymes (ectopeptidases) are integral membrane proteins, orientated asymmetrically with the catalytic site exposed to the extracellular surface, which enables a versatile range of physiological and pathological functions. Ectopeptidases may regulate the release of many growth factors and their receptors into the circulation, as well as activating or inactivating circulating signalling molecules, thereby regulating the availability of ligands for the corresponding receptors. Additionally, many of these ectopeptidases have functions not limited to proteolysis, but are able in themselves to function as receptors, transducing intracellular signals. A versatile range of functions, such as the modulation of cell-signalling, matrix degradation, cell adhesion and migration, which are particularly important for tumour cell growth and dissemination, are attributed largely to the ectopeptidases. Even a minor disruption in the normal proteolytic equilibrium can influence tumor progression, and a range of ectopeptidases, including neutral endopeptidase 24.11, aminopeptidase N, dipeptidyl peptidase IV, angiotensin-converting enzyme, and the disintegrin-metalloproteinases, have been shown to be involved in tumour development and metastasis. The ability to degrade and inactivate peptide hormones and growth factors, with the resultant modulation of the tumour-host interface, may play an important role in the pathogenesis, development or progression of a range of cancers, and the extracellular orientation of the ectopeptidases makes them particularly accessible, and therefore interesting, with regard to therapeutical applications.

Key words: NEP, APN, DPIV, ACE, GPCR, EGFR transactivation

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Introduction

It has long been recognized that the expression pattern of proteases may be changed in malignant tumours, indicating a putative involvement in tumour development and tumour growth. Their roles in cancer progression and invasion are evidenced by the ability to influence proliferation, angiogenesis, tumour cell migration, and metastatic behaviour. Neutral endopeptidase 24.11 (NEP, CD10), aminopeptidase N (APN, CD13), dipeptidyl peptidase IV (DPIV, CD26) and angiotensin-I converting enzyme (ACE, CD143), and the disintegrin-metalloproteinases, ADAM9, ADAM12 and ADAM15, belong to a large group of multifunctional, extracellularly orientated, membrane-bound proteolytic enzymes classified as ectopeptidases, which have all been shown to participate in the post-secretory processing of neuropeptides, peptide hormones and growth factors. They are all widely distributed and have been found in various different cell types of different organs and tissues, including benign and malignant tumours (Nanus et al., 1997; Antczak et al., 2001). Each ectopeptidase has the potential to possess proteolytic, adhesion, and signalling abilities, enabling a versatile range of physiological and pathological functions. Tumour cell growth, invasion, and metastasis depend on timely balanced changes of proteolytic activity and cell-cell and cell-matrix interactions, which could be influenced by the activity of these ectopeptidases.

The ectopeptidases in tumour biology

The accumulation of mutations during carcinogenesis results in six essential alterations in cell physiology that collectively dictate malignant growth: self sufficiency in cell growth, insensitivity to growth-inhibitory signals, evasion of cell death, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan and Weinberg, 2000). Most of these alterations affect cell signalling pathways, and the majority of oncogenes and tumour suppressor

genes are integral components of cellular signalling circuits, which are up-regulated or constitutively activated in malignant cells. However, the true initiators of these circuits are the extracellularly-derived signalling molecules, which may be secreted by the host cells or the tumour cell itself.

Autocrine, paracrine and juxtacrine modulation of cell signalling by growth factors, cytokines, hormones and signalling peptides plays a key role in the promotion of proliferation, inhibition of apoptosis, and facilitation of invasion and migration through tissues, as well as the induction of angiogenesis. Synthesis and/or amplified secretion of growth factors and regulatory peptides is often a feature obtained during carcinogenesis, even of non-endocrine tumours, and a single tumour may express a number of different autocrine or paracrine loops to maintain malignant growth. Both autocrine and paracrine loops have been observed in most cancers, and these cell signalling pathways are frequently composed of three main aspects. Extracellular signalling molecules bind to cell-surface receptors, which activate intracellular circuits to initiate the cellular effect (Fig. 1). Alterations in any of these pathway components may give rise to tumour biology-relevant modifications in cell signalling and affect the sensitivity of the tumour cell to external stimuli: changes to the availability of extracellular signalling molecules by increased or decreased synthesis, to the transcellular transducers of those signals, such as the receptor molecules, or to the functioning of the intracellular circuits by structural changes to molecular components.

While the majority of molecular analyses in cancer searched for changes in the intracellular circuits, little is known about the biological function of cell surface molecules that modulate the immediate cellular environment, such as the availability of the extracellular signalling molecules. Many of these signalling pathways involve the extracellular regulation of ligand availability through proteolysis, which may be mediated by cell membrane-bound proteolytic enzymes (ectopeptidases) expressed on the surface of tumour or host cells.

Cell membrane-bound proteolytic enzymes (ectopeptidases) are multifunctional membrane proteins, which are widely distributed among various cell systems. Ectopeptidases are integral membrane proteins, orientated asymmetrically with the catalytic site exposed to the extracellular surface, which enables a versatile range of physiological and pathological functions, ranging from proteolytic 'shedding' of signalling molecules, degradation of the extracellular matrix and tissue remodelling, to adhesion and cell migration, and the transduction of specific intracellular signals and involvement in inflammation.

Proteases may regulate the release of many growth factors and their receptors into the circulation, as well as activating or inactivating circulating signalling molecules. Additionally, many proteases have functions not limited to proteolysis, but are able in themselves to function as receptors, transducing intracellular signals. The individual ectopeptidases are each able to perform several, often overlapping functions, and, as a result, the expression of each ectopeptidase must be precisely regulated, in a tissue- and cell-specific manner. Even a minor disruption in the normal proteolytic equilibrium can influence the development of inflammatory and autoimmune diseases (Bank et al., 2000). Functions, such as cell-signalling, matrix degradation, cell adhesion and migration, which are particularly important for tumour cell growth and dissemination have been reported for a number of ectopeptidases.

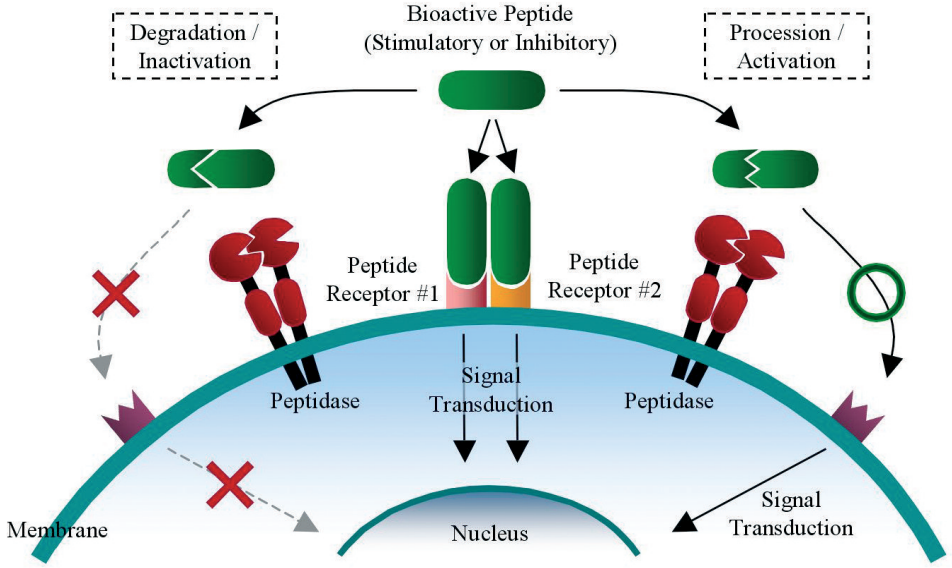

Fig. 1. Ectopeptidase modulation of signal transduction pathways. Inactivation or activation of extracellular signalling molecules by ectopeptidases may modulate intracellular signalling pathways by regulating the availability of ligands for their receptors, of which there may be a varied range, often with more than one subtype (modified from Ino et al., 2004).

Neutral endopeptidase 24.11 (NEP)

Neutral endopeptidase 24.11 (NEP, neprilysin, enkephalinase, CD10, EC 3.4.24.11) is a 90-110 kDa zinc-dependent metallopeptidase belonging to the gluzincin family of metallopeptidases. The type II integral membrane protein is identical to the common acute lymphoblastic leukaemia antigen (CALLA). The 80kb NEP gene is located on chromosome 3q21-27 (Barker et al., 1989), and the transcribed mRNAs range from 2.7 to 5.7 kb and exhibit tissue-specific and developmentally regulated expression (Li et al., 1995). The encoded 749 amino acids are inserted asymmetrically into the membrane, with the large extracellular C-terminal catalytic domain anchored by the 23 amino acid transmembrane region and the short 27 amino acid cytoplasmic N-terminal tail (Crine et al., 1997). The human NEP sequence contains 12 cysteine residues that may possibly form stabilizing disulfide bridges in the active enzyme, and 5 glycosylation sites, which are important for transport to the cell surface and full enzyme activity (Lafrance et al., 1994). Usually found in homodimeric conformation, NEP cleaves peptide bonds on the amino side of hydrophobic residues, but also has peptidyl-dipeptidase activity with certain substrates (Roques et al., 1993). The endogenous substrates of NEP include the enkephalins, atrial natriuretic peptides, substance P, and other tachykinins, as well as a wide range of other bioactive peptides (Matsas et al., 1984; Kenny, 1993), such as somatostatin, neurokinin, cholecystokinin-8, angiotensin I, angiotensin II, bradykinin, gastrin-releasing peptide, calcitonin, calcitonin gene-related peptide, interleukin-1, bombesin, and endothelin-1. NEP is expressed in various tissues (Kenny, 1993), including immune cells, the brush border membranes of the kidney, intestine, and placenta, the brain, thyroid, lung and prostate, where it regulates proliferation and differentiation by degrading signalling peptides (Crine et al., 1997). The association of NEP with acute lymphoblastic leukaemia, Alzheimer's disease, multiple sclerosis, asthma, inflammation, hypertension, and neoplastic transformation and progression is assumed to be due to the deregulation of peptide processing (Letarte et al., 1997; Sumitomo et al., 2005).

Of the ectopeptidases, predominantly NEP has been associated with peptide-mediated proliferation. There is ample evidence for the growth retarding effects of NEP, with the effect of NEP being mediated by regulatory peptides and peptide hormones (Shipp et al., 1991a). In the lung, NEP regulates growth and maturation by inactivating the mitogenic activity of bombesin-like peptides (King et al., 1993), and down-regulation of NEP occurs frequently in lung cancer (Shipp et al., 1991b). Furthermore, the expression of NEP correlates inversely with tumour cell proliferation of small cell (Shipp et al., 1991b) and non-small cell lung cancers (Ganju et al., 1994). Similarly, in androgen-independent prostate cancer, NEP inhibits cancer progression by inactivating growth-promoting peptides (Papandreou et al., 1998), promoting apoptosis (Sumitomo et al., 2000a) and inhibiting migration (Sumitomo et al., 2000b).

Indeed, in the liver, the overall expression pattern of NEP corresponds to proliferation and/or differentiation of hepatocytes. NEP is generally expressed by bile canaliculi in normal liver and many HCCs (Chu and Arber 2000; Borscheri et al., 2001; Chu et al., 2002). However, NEP is only occasionally expressed by foetal hepatocytes (Röcken et al., 2004). The association between proliferation and NEP expression in the liver has been evaluated by comparing the proliferation indices (number of Ki-67-positive nuclei) with expression of NEP (Röcken et al., 2004), and it was shown that, overall, the expression of NEP in non-neoplastic and neoplastic hepatocytes correlates inversely with their state of proliferation or differentiation (Röcken et al., 2004).

Up-regulation of NEP has been reported in melanomas (Carrel et al., 1993) and in gastric adenocarcinomas (Carl-McGrath et al., 2004). This may reflect a regulatory response to excessive growth, resulting from exposure to trophic peptides (Letarte et al., 1997). However, *in vitro* experiments have shown that inhibition of NEP activity retards cell growth in liver and gastric cancer cell lines (Carl-McGrath et al., 2004; Röcken et al., 2004), contradicting the growth inhibiting effect of NEP. Interestingly, NEP also exhibits a preferentially cytoplasmic location in prostate cancer cells (Renneberg et al. 2001) and diffuse-type gastric carcinomas, which has been attributed to alterations in intracellular targeting (Gomes et al., 2003). This may indicate that the function of NEP in cancers is not only related to its extracellularly-orientated peptidase function.

Aminopeptidase N (APN)

Aminopeptidase N (APN, CD13, EC 3.4.11.2), another member of the gluzincin family of metal-dependent proteases, is an approximately 150 kDa type II transmembrane protease encoded by the 35 kb APN gene, located on chromosome 15q25-26 (Noren et al., 1997). The 967 amino acid sequence contains a single 24 amino acid transmembrane segment near the 8-10 amino acid cytoplasmic N-terminal. APN contains ten N-glycosylation sites and exists as a homodimer. A 40 amino acid stalk connects the transmembrane segment to the catalytic domain, which consists of two subunits. The C-terminal subunit is assumed to bind substrates, while the N-terminal subunit contains the HELAH zinc-binding motif in the single catalytic site, preferentially cleaving N-terminal unsubstituted neutral amino acids from oligopeptides (Noren et al., 1997). APN cleaves vasoactive peptides, such as angiotensin III and kallidin, neuropeptides, such as enkephalins and endorphins, and chemotactic peptides, such as MCP-1, as well as neurokinin A, somatostatin, and interleukin-8, but is unable to cleave bradykinin or substance P, which act as

endogenous inhibitors. The broad substrate specificity reflects its wide expression pattern (Noren et al., 1997). APN is found in the brush border membranes of intestine and kidney, on the synaptic membranes of the central nervous system, and on the surface of macrophages, granulocytes, and lymphocytes, as well as on endothelial cells, smooth muscle cells, and fibroblasts. In addition to its role in the regulation of cell growth and differentiation by modulating local peptide concentrations, APN is involved in antigen processing and presentation (Larsen et al., 1996), serves as receptor for the human coronavirus 229E (Yeager et al., 1992), transduces intracellular signals via MAP kinases (Lendeckel et al., 1998), and mediates invasion and metastasis in a range of tumours and cell lines (Menrad et al., 1993; Saiki et al., 1993; Fujii et al., 1995) via initiation of collagen IV degradation (Saiki et al., 1993). APN is also associated with neoangiogenesis (Pasqualini et al., 2000; Bhagwat et al., 2001, 2003).

The expression and putative pathophysiological role of APN has been studied in many different malignant tumours (Mechtersheimer and Moller, 1990; Tokuhara et al., 2001). APN is up-regulated in melanomas (Menrad et al., 1993), colon cancer (Hashida et al., 2002) and various tumour cell lines, including those obtained from renal cell carcinomas (Gohring et al., 1998; Stange et al., 2000), prostate cancer (Ishii et al., 2001b), choriocarcinoma (Ino et al., 1994), melanoma (Menrad et al., 1993), fibrosarcoma (Fujii et al., 1996), osteosarcoma (Kido et al., 1999), and leukaemia (Mishima et al., 2002).

The expression of APN has been linked to tumour cell proliferation, degradation of extracellular matrix and metastatic behaviour (Menrad et al., 1993; Kido et al., 1999; Ishii et al., 2001a; Hashida et al., 2002). Almost all of these biological effects were attributed to the ectopeptidase activity, and the deregulation of the local balance of peptide and growth factor activation/inactivation. APN may mediate its pathophysiological effect by cleaving regulatory peptides, such as bradykinin, enkephalins, or somatostatin, so promoting tumour cell proliferation. APN can have opposing effects on cell proliferation of both hepatoma and gastric cancer cells, which may reflect the involvement of APN in different pathways (Carl-McGrath et al., 2004; Röcken et al., 2004). Interestingly, previous reports have demonstrated that APN also mediates IL-8-induced apoptosis of leukaemia cell lines (Mishima et al., 2002).

In addition to proliferation and apoptosis, APN has been shown to be involved in invasion. The invasive potential of rodent and human tumour cells could be significantly inhibited by anti-APN antibodies or peptide inhibitors of APN enzymatic activity (Menrad et al., 1993; Saiki et al., 1993; Fujii et al., 1995; Kido et al., 1999). These results were paralleled by anti-sense strategies (Kido et al., 2003), and are believed to be due to proteolytic degradation of and adhesion to the extracellular matrix. In a study of gastric cancers, APN was found in a majority of the lymph node metastases, often demonstrating high intensity staining in over 50% of the cells (Carl-McGrath et al., 2004). Although no correlation was found with lymph node status of the tumours in this study, the role of APN in metastasis is supported by the results of various investigations. In colon and pancreatic carcinoma patients, survival is significantly lower in patients with APN-positive tumours (Hashida et al., 2002; Ikeda et al., 2003), especially in patients already exhibiting lymph node metastasis (Hashida et al., 2002). These studies were confirmed by clinical studies using Bestatin, an aminopeptidase inhibitor, which has been shown in a range of cell lines to inhibit proliferation and induce apoptosis (Sekine et al., 2001). In the treatment of stomach cancer (Niimoto and Hattori 1991), the cancer patients receiving Bestatin showed higher survival rates than the control groups, particularly in patients exhibiting deeper tumour invasion. Additionally, treatment with Bestatin reduced the incidence of returning peritoneal dissemination. One of the factors essential for successful distant metastasis is angiogenesis, and APN has been identified as a specific marker of neoangiogenic vasculature endothelial cells (Pasqualini et al., 2000). Additionally, blocking APN activity inhibited capillary network formation and reduced tumour growth in animal models (Bhagwat et al., 2001, 2003).

Dipeptidyl peptidase IV (DPIV)

Dipeptidyl peptidase IV (DPIV, CD26, EC 3.4.14.5) is a multifunctional type II cell surface glycoprotein with a molecular mass of approximately 110 kDa. The human DPIV gene is located on the long arm of chromosome 2q24.2 and covers 82 kb (Gorrell et al., 2001). The predicted protein of 766 amino acids, with six amino acids in the cytoplasmic region and a 22 residue hydrophobic transmembrane domain, contains nine potential N-linked glycosylation sites, and has an α/ß hydrolase domain and a seven-blade ß-propeller domain, characteristic of members of the prolyl oligopeptidase gene family. DPIV may be cell bound or soluble, occuring in the serum (sDPIV). Cell-associated DPIV is widely expressed on T cells, B cells, natural killer cells, endothelial cells and epithelial cells. DPIV has three different functions: adenosine deaminase (ADA) binding, serine peptidase activity, and extracellular matrix (ECM) binding. These different biological activities of DPIV and its ubiquitous expression may reflect its diverse, sometimes opposing functions in physiological and pathological settings.

It has long been recognized that the expression pattern of DPIV is changed in malignant tumours, indicating a putative involvement in tumour development and tumour growth. DPIV was found to be up-regulated in T-cell lymphoblastic lymphoma, thyroid cancer, adenocarcinoma of the lung and basal cell carcinomas of the skin, and to be down-regulated in

malignant melanomas (Dang and Morimoto, 2002; Pro and Dang, 2004). DPIV is also significantly up-regulated on the mRNA and protein level in HCCs and is expressed by two hepatoma cell lines (Röcken et al., 2004). Serum levels of DPIV are increased in humans and animals suffering from HCCs or hepatomas (Hanski et al., 1986; Gorrell et al., 2001), and the expression pattern of DPIV in HCCs and cirrhotic livers is different from that of non-cirrhotic livers (Matsumoto et al., 1992; Stecca et al., 1997). Serine peptidase activity of DPIV reverses malignant transformation of malignant melanomas (Wesley et al., 1999) and prolongs survival and decreases invasive activity of ovarian carcinoma cell lines (Kajiyama et al., 2003). The enzymatic activity may contribute, but is not essential for DPIV-mediated signal transduction (Morimoto and Schlossman, 1998; von Bonin et al., 1998), and signal transduction via DPIV affects proliferation of T-cell lymphomas (Kähne et al., 1999), and influences hepatocarcinogenesis (Gaetaniello et al., 1998), by activating tyrosine kinases and thereby inducing apoptosis in hepatoma cell lines (Gaetaniello et al., 1998). DPIV has been previously detected in well- and moderately-differentiated gastric cancers, but weakly or not at all in poorly differentiated gastric cancers (Sakamoto et al., 1993), a finding not confirmed by another study (Carl-McGrath et al., 2004). Interestingly, DPIV inhibition significantly increased gastric cancer cell proliferation (Carl-McGrath et al., 2004), but unexpectedly retarded cell growth in hepatoma cell lines (Röcken et al., 2004), which suggests that DPIV may be involved in the pathology of gastrointestinal carcinomas in different ways in different tumours

Angiotensin-Converting Enzyme (ACE)

Angiotensin-converting enzyme (ACE, CD143, EC 3.4.15.1) is a 150-180 kDa type I integral membrane protein with 17 potential N-linked glycosylation sites (Soubrier et al., 1988). The human ACE gene covers 21 kb on chromosome 17q23, and encodes 1306 amino acids, consisting of a 28 residue C-terminal cytoplasmic domain, a 22 amino acid transmembrane anchor, and the extracellularly-orientated carboxypeptidase domain. ACE is a unique metallopeptidase in that it has two functionally active catalytic sites (Soubrier et al., 1988), probably due to gene duplication (Skidgel and Erdos, 1987). Each site displays differences in affinity for substrates and inhibitors (Georgiadis et al., 2003). A testis-specific soluble isoform of ACE, generated by alternative splicing, has only one catalytic site, and corresponds to the C-terminal region of full length ACE (Ehlers et al., 1992). Both isoforms of ACE are transcribed from the gene by two alternative promoters (Howard et al., 1990; Hubert et al., 1991). Another soluble form of ACE is derived from the membrane-bound protein by proteolytic cleavage of the membrane-inserted C-terminal stalk (Ehlers and Riordan, 1990), and there is evidence for the secretion of an alternative splicing variant lacking the transmembrane domain (Sugimura et al., 1998). ACE is almost ubiquitously expressed. Apart from being expressed on the luminal surface of endothelial cells in vascular tissues (Igic and Kojovic, 1980), ACE is also found on epithelial cells in the brush border of the proximal tubule of the kidney, the small intestine and the placenta (Igic et al., 1977; Johnson et al., 1984), as well as in neuroepithelial and vascular smooth muscle cells, and fibroblasts and macrophages (Igic and Behnia, 2003). Considerable amounts of ACE are expressed by epithelial cells in the gastrointestinal tract, predominantly in the intestinal mucosa, but also in the chief cells of the gastric foveolar epithelium (Kobayashi et al., 1991; Laliberte et al., 1991; Nonotte et al., 1993, 1995; Carl-McGrath et al., 2004), where it may play a role in the metabolism of gastrointestinal hormones and regulatory peptides (Turner et al., 1987; Lendeckel et al., 2000).

ACE plays a major role in the regulation of blood pressure, cleaving angiotensin I to generate the hypertensive angiotensin II, the major effector peptide of the renin-angiotensin system, and inactivating the hypotensive bradykinin (Re, 2004). Local angiotensin II-generating systems are believed to be responsible for the blood pressure-independent effects of renin-angiotensin system inhibitors. Angiotensin II is involved in the regulation of cell proliferation via its G-protein coupled receptors (GPCR), type 1 (AT1) and type 2 (AT2) (de Gasparo et al., 2000; Suzuki et al., 2003), and can induce the metalloproteinase-mediated secretion of EGF-like growth factors (Schafer et al., 2004), and the triple-membrane passing signal for EGFR transactivation (Mifune et al., 2005; Olivares-Reyes et al., 2005).

Upregulation of angiotensin II and its precursors has been observed in connection with stress-induced ulcers and gastric lesions (Seno et al., 1997; Yee et al., 1997; Mou et al., 1998) and the inhibition of ACE in animal models reduces the incidence and severity of stress-induced gastric ulcers (Bailey et al., 1987; Bhounsule et al., 1990; Bhandare et al., 1992; Ender et al., 1993; Cullen et al., 1994; Rao et al., 1995; Uluoglu et al., 1998). ACE cleaves several gastrointestinal regulatory peptides and peptide hormones, and the secretion of these signalling peptides is a necessary part of the response to gastric mucosal damage. Many of these peptides have been shown to alleviate or inhibit the extent of stress-related gastric lesions (Hernandez et al., 1983; Mercer et al., 1997; Brzozowski et al., 1999; Konjevoda et al., 2001), and cleavage of these peptides by ACE may result in attenuation of their bioactivity.

Being a relatively non-specific enzyme, ACE also cleaves di- or tripeptides from a number of synthetic and naturally occurring substrates, including substance P, opioid peptides (Met-enkephalin-Arg^6-Phe^7, heptapeptide, ß-neoendorphin, dynorphin1-8, dynorphin1-6), neurotensin, chemotactic peptide, luteinizing hormone releasing hormone, cholecystokinin-8, [Leu15]gastrin11-17, and B-chain of insulin. This wide range of substrates may explain the

involvement of ACE in a variety of physiological and pathological processes, such as neovascularization (Volpert et al., 1996), fertilization (Krege et al., 1995), atherosclerosis (Metzger et al., 2000), kidney and lung fibrosis (Nguyen et al., 1994; Metzger et al., 1999; Leehey et al., 2000), smooth muscle and myocardial hypertrophy (Naftilan et al., 1989; Aceto and Baker, 1990), and inflammation and wound healing (Sun and Weber, 1996).

A polymorphism of the ACE gene, comprising an insertion (I) or deletion (D) of a 287-bp DNA fragment in intron 16, leads to variances in ACE expression and activity in blood and tissues of affected individuals (Rigat et al., 1990; Tiret et al., 1992; Villard et al., 1996), with individuals harboring the DD genotype exhibiting increased activity of ACE in blood and tissues. Previous studies have linked this polymorphism to various malignancies, including renal, prostate and breast cancer (Usmani et al., 2000; Koh et al., 2003; Medeiros et al., 2004). The ACE has also been shown to play a role in the development of early gastric cancers (Ebert et al., 2005).

Observations made in retrospective cohort studies suggested that ACE-inhibitors decrease the risk of cancer (Lever et al., 1998), including those of the liver (Friis et al., 2001). In addition to epidemiological studies supporting the involvement of the angiotensin II system in cancer progression (Lever et al., 1998), there is also strong experimental evidence that this system plays an important role in tumour biology, influencing tumour cell proliferation (Reddy et al., 1995; Yasumaru et al., 2003), the remodelling of the interstitial matrix (Suzuki et al., 2003), the local peritumorous inflammatory reactions (Smith and Missailidis, 2004), neoangiogenesis (Yoshiji et al., 2001a, 2002b; Fujita et al., 2002), and metastatic behaviour (Röcken et al. 2005).

The local angiotensin system was shown to be strongly involved in matrix remodelling. Both inhibition of ACE activity by ACE-inhibitors and blockade of the angiotensin-II type 1 receptor significantly attenuated the development of liver fibrosis (Jonsson et al., 2001). HCCs, in turn, often occur in cirrhotic livers and progression requires degradation and remodeling of the surrounding matrix (Theret et al., 2001). The binding of angiotensin II to the angiotensin-II type 1 receptor has a trophic and mitogenic effect on cell growth. Angiotensin-II induces dose-dependent vascular endothelial growth factor (VEGF), which in turn correlates with tumour progression of HCCs (Torimura et al., 1998). ACE may also be involved in tumour progression through the conversion of angiotensin I to angiotensin II, which induces neovascularization (Tamarat et al., 2002), and inhibition of ACE activity by captopril inhibits angiogenesis and slows tumour growth in rats (Volpert et al., 1996). The function of ACE in metastasis has generally been attributed to the promotion of angiogenesis (Yoshiji et al., 2001b, 2002a; Fujita et al. 2002). However, the ACE insertion/deletion gene polymorphism is also associated with nodal status in gastric cancers (Röcken et al., 2005), with the DD genotype being significantly correlated with a greater number of lymph node metastases and advanced UICC tumor stage than the ID or II genotype.

The ADAMs (A Disintegrin And Metalloproteinase)

Instead of degrading bioactive peptides and peptide hormones, another group of ectopeptidases are better known for their roles in the shedding of membrane-bound growth factors or receptors. The ADAMs (A Disintegrin And Metalloproteinase) are a family of membrane-anchored, cell-surface glycoproteins, containing pro-, metalloprotease, disintegrin (RGD-binding motif), cysteine-rich, epidermal growth factor (EGF)-like, transmembrane and cytoplasmic domains. Removal of the prodomain occurs during transport through the secretory pathways of the cell, with the mature, proteolytically active form being expressed on the cell surface. After removal of the prodomain and its cysteine switch, the metalloproteinase domain is proteolytically active, enabling the shedding or degradation of a wide range of substrates. Both the disintegrin and cysteine-rich domains bind adhesion molecules, such as integrins and syndecans. The EGF-like domain may play a role in the association between the ADAM and the EGF-like ligands to be shed. The cytoplasmic tail is involved in intracellular signalling via Src-homology-3 (SH3) binding motifs.

ADAMs are able to modulate cellular adhesion to adjacent cells and extracellular matrix by binding to adhesion molecules - a prerequisite for expansion, invasion and spreading of tumour cells. The presence of an RGD motif in a ß-loop structure of the disintegrin domain of ADAM9 and 15 facilitates binding to the integrins α6ß1, α9ß1, αvß3, or αIIß3 (Tselepis et al., 1997; Zhang et al., 1998; Nath et al., 1999, 2000; Eto et al., 2002; Tomczuk et al., 2003), and ADAM-integrin interactions have been implicated in tissue and matrix remodelling (Arndt et al., 2002; Le et al., 2003).

In particular, the ADAMs metalloprotease activity influences cell proliferation. In the stomach, proliferation and migration of gastric epithelial cells, and the cellular response to infection and mucosal injury are regulated, in part, by the EGFR transduction pathway (Miyazaki et al., 1996, 2001; Zushi et al., 1997; Keates et al., 2001; Chen et al., 2002; Wallasch et al., 2002), which plays a key role in tumour proliferation, angiogenesis, invasion and metastasis (Jonjic et al., 1997; Eccles, 2000; Fischer et al., 2003). Transactivation of the EGFR by hormones and regulatory peptides that are found in the gastrointestinal tract, such as gastrin, angiotensin II, bradykinin, bombesin, or substance P, is apparently mediated by disintegrin-metalloproteinase shedding of EGFR-ligands (Tsutsui et al., 1997; Dong et al., 1999; Miyazaki et al., 1999; Asakura and Kitakaze, 2002; Fisher et al., 2003; Schafer et al., 2004). Shedding can be induced by various activating and pathological stimuli, and the specific ligand released is defined by

cellular context and stimulus. There is compelling evidence that ADAM9, 12 and 15 can release some of the transmembrane protein-derived EGFR ligands, such as HB-EGF, amphiregulin, and TGFα (Izumi et al., 1998; Asakura et al., 2002; Schafer et al., 2004). Up-regulation of these ADAMs could increase ligand-shedding, and thereby the availability of EGF-like ligands for the EGFR, and, like overexpression of the EGFR ligands (Ruck and Paulie 1997, 1998), promote transformation and proliferation by autocrine mechanisms.

ADAM9

ADAM9 (MDC-9, meltrin γ) is expressed in a wide range of tissues (Weskamp et al., 1996). Shed substrates include heparin-binding epidermal growth factor (HB-EGF), ß-amyloid precursor protein, fibronectin, ß-casein, gelatin, TNFα, p75 TNF receptor and c-kit ligand-1 (Izumi et al., 1998; Roghani et al., 1999). The ECD integrin binding motif within the disintegrin domain mediates binding to integrin α6ß1, resulting in enhanced cellular motility (Nath et al., 2000). The disintegrin domain also mediates the binding of ADAM9 to another integrin, αvß5 (Zhou et al., 2001). The cytoplasmic tail contains potential SH3-binding motifs, to which the adaptor proteins endophilin I and SH3PX1 are assumed to bind (Howard et al., 1999). The cytoplasmic tail can also be phosphorylated by protein kinase Cδ, which may activate ADAM9-mediated HB-EGF-shedding (Izumi et al., 1998; Gechtman et al., 1999). Increased expression of ADAM9 has been detected in breast cancer (O'Shea et al., 2003; Lendeckel et al., 2005), in pancreatic ductal adenocarcinoma (Grutzmann et al., 2003), gastric cancer (Carl-McGrath et al., 2005), and liver cancer (Le et al., 2003; Tannapfel et al., 2003). Interestingly, it has been suggested that differential processing or post-translational modification of the ADAM9 proteins may occur in breast carcinomas compared to non-neoplastic tissue (O'Shea et al., 2003). Expression of ADAM9 was also detected in prostate adenocarcinomas and tumour cell lines (Karan et al., 2003).

ADAM12

ADAM12 (meltrin α) is broadly expressed in a variety of tissues. There are two alternate forms of ADAM12, created by alternative splicing (Gilpin et al., 1998). The longer form (ADAM12-L) produces a transmembrane protein, whereas the shorter form lacks the transmembrane and cytoplasmic domains (ADAM12-S) and transits through the endomembrane system to be secreted (Hougaard et al., 2000; Kadota et al., 2000; Cao et al., 2002). In addition to the shedding of HB-EGF (Asakura et al., 2002), soluble ADAM12 degrades insulin-like growth factor (IGF) binding proteins 3 and 5, thereby increasing the available pool of IGF-1 and -2 (Loechel et al., 2000). Although lacking a defined integrin-binding motif within the disintegrin domain, ADAM12 binds to α9ß1 integrin (Eto et al., 2000), as well as regulating ß1 integrin function (Kawaguchi et al., 2003). ADAM12 also supports cell adhesion and migration through the interaction of its cysteine-rich domain with syndecans (Iba et al., 2000; Thodeti et al., 2003). The SH3-binding motifs in the cytoplasmic tail of ADAM12 are assumed to mediate binding to Src, Yes, and Grb2 (Kang et al., 2000; Suzuki et al., 2000) and p85α, a regulatory subunit of PI 3-kinase (Kang et al., 2001), indicating its role in the activation of intracellular signalling pathways. In keeping with its important role in myoblast fusion (Yagami-Hiromasa et al., 1995), the cytoplasmic tail also binds to the muscle specific actin-binding proteins, α-actinin-1 and -2 (Galliano et al., 2000). Increased expression of ADAM12 has been detected in giant cell bone tumours (Tian et al., 2002), in breast cancers and cell lines (Lendeckel et al., 2005), and in liver cancer (Le et al., 2003; Tannapfel et al., 2003). Treatment with anti-ADAM12 antibodies has been shown to enhance the proliferation of gastric cancer cell lines (Carl-McGrath et al., 2005). Interestingly, the level of expression of ADAM12 was significantly lower in diffuse-type, compared with intestinal-type gastric carcinomas. Down-regulation of adhesion molecules has been frequently observed in diffuse-type carcinomas (Tahara 2004), including syndecan-1 (Watari et al., 2004) and ß1-integrin (Solcia et al., 1996), adhesion molecules involved in ADAM12-mediated formation of focal adhesions and cell spreading (Iba et al., 1999; Thodeti et al., 2003). The lower expression of ADAM12 in diffuse-type may reflect the different pattern of tumour-host interactions that distinguishes diffuse- from intestinal-type gastric cancers (Carl-McGrath et al., 2005).

ADAM15

ADAM15 (MDC-15, metargidin) also exhibits a wide expression pattern. ADAM15 is involved in the lysophosphatidic acid-induced EGFR transactivation (Schafer et al., 2004), and mediates the shedding of amphiregulin and TGFα (Schafer et al., 2004), as well as being involved in the degradation of type IV collagen and gelatin (Martin et al., 2002). ADAM15 is the only member of the ADAMs family to contain the well-known RGD-integrin binding motif within the disintegrin domain, and can bind to αvß3 and α5ß1 integrins (Nath et al., 1999). Binding to integrin α9ß1 also occurs via the disintegrin domain, but in an RGD independent manner (Eto et al., 2000, 2002). With SH3 binding motifs and potential phosphorylation sites in the cytoplasmic tail, ADAM15 associates with the adaptor proteins endophilin I, SH3PX1 and Grb2, and the tyrosine kinases Src, Lck and Hck (Howard et al., 1999; Poghosyan et al., 2002; Yasui et al., 2004). ADAM15 is up-regulated in lung carcinomas (Schutz et al., 2005), high expression levels of ADAM15 have been reported in cell lines derived from haematological malignancies

(Wu et al., 1997), and over-expression of ADAM15 reduces ovarian cancer cell adhesion and motility (Beck et al., 2005). ADAM15 may also play a role in pathological neovascularization (Horiuchi et al., 2003). Additionally, elevated transcription of ADAM15 (Yoshimura et al., 2002) and upregulation on both RNA and protein level (Carl-McGrath et al., 2005) has been detected in gastric cancer compared with non-neoplastic tissue. ADAM15 has also been shown to influence gastric cancer cell proliferation (Carl-McGrath et al., 2005).

Ectopeptidases as therapeutic targets

In summary, accumulating evidence associates a differential expression of individual ectopeptidases with various types of cancer on both the transcriptional and protein level. There are various, often conflicting explanations for the observed cancer-related up- or down-regulation of these ectopeptidases, and differential expression of the ectopeptidases may contribute to the development and/or progression of cancers in different ways. It has been demonstrated that the inhibition of the ectopeptidase activity affects tumour cell proliferation in different ways, being able to both activate and inactivate *in vitro* proliferative processes. Ectopeptidases have broad, partially overlapping substrate specificities, and their expression pattern is not generally uniform, with different ectopeptidases cleaving the same substrate at different anatomical sites. The deregulation of the subtle balance between interactions with adhesion molecules and the extracellular matrix, and the shedding of surface proteins including growth factors and their receptors, affects inter- and intracellular signalling pathways. Indeed, the maintenance of malignant growth is dependent on an extremely complex system of proteolytic regulation. In particular, the triple membrane passing signal concept of EGFR transactivation, which involves the GPCR stimulation of ADAMs-mediated shedding of EGFR ligands, is becoming more and more established (Wallasch et al., 2002). As shown in Figure 2, the ectopeptidases described here are involved in the regulation of the local GPCR ligand concentration (NEP, APN, DPIV, ACE), as well as regulating the release of the EGFR ligands from the cell surface (ADAM9, ADAM12, ADAM15).

The ability to degrade and inactivate peptide hormones and growth factors, with the resultant modulation of the tumour-host interface, may play an important role in the pathogenesis, development or

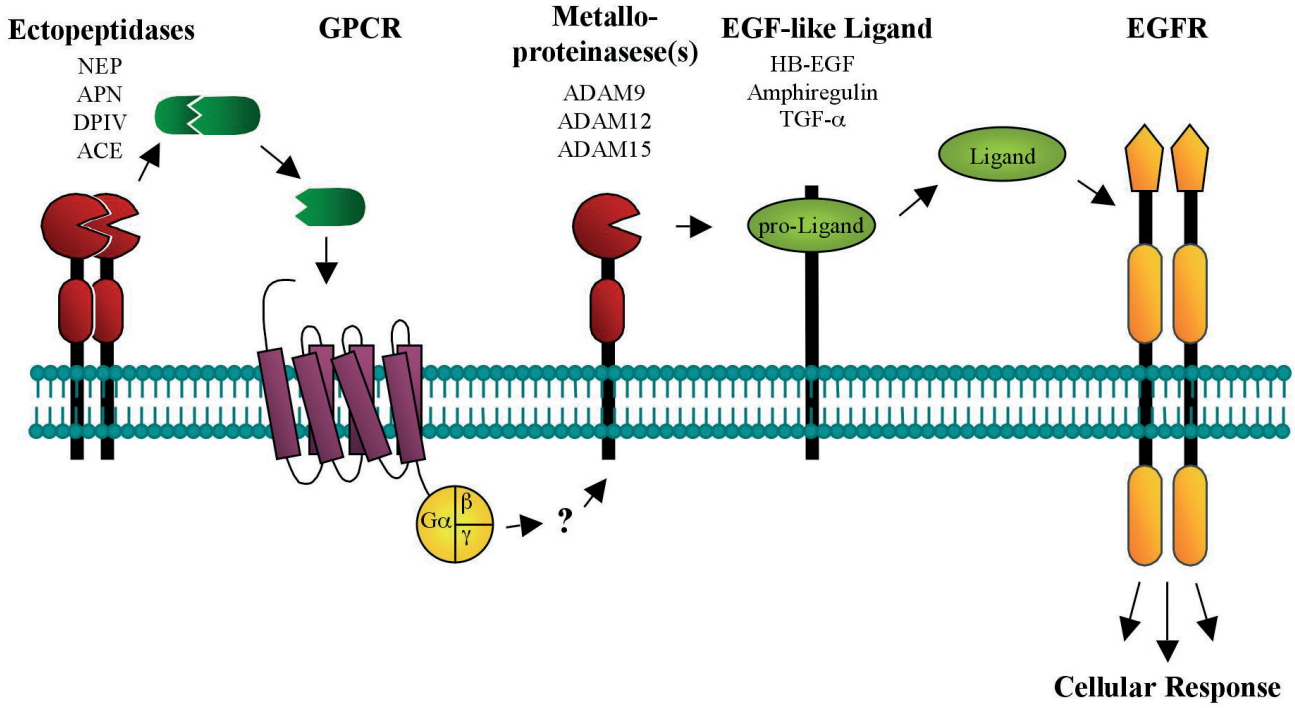

Fig. 2. The role of the ectopeptidases in epidermal growth factor receptor transactivation. The triple membrane passing signal concept of epidermal growth factor receptor (EGFR) transactivation involves the stimulation of G-protein coupled receptors (GPCR) by GPCR ligands, which induces the ADAMs-mediated shedding of EGFR ligands. Ectopeptidases are involved in the regulation of the local GPCR ligand concentration (NEP, APN, DPIV, ACE), as well as regulating the release of the EGFR ligands from the cell surface (ADAM9, ADAM12, ADAM15) (modified from Fischer et al., 2003).

progression of a range of cancers. The extracellular orientation of the ectopeptidases makes them particularly accessible, and therefore interesting, with regard to therapeutical applications. Ectopeptidase inhibitors have been suggested as treatment for cancer (Antczak et al. 2001), and the aminopeptidase inhibitor, Bestatin, is currently being studied in clinical trials (Ichinose et al., 2003; Ota and Uzuka, 1992). ACE inhibitors and AT1 blockers are already used to treat hypertension. However, although the deregulation of the ectopeptidases leaves no doubt that they are important for tumour cell biology, the use of ectopeptidase inhibitors, particularly when orally administered, may be contraindicated in some cancer patients. In those cancers where the up-regulation of the ectopeptidases is an internal response to uncontrolled growth, treatment with ectopeptidase inhibitors may increase the proliferative effect mediated by systemic neuropeptides and growth factors.

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Accepted June 8, 2006