

Ectopeptidases in pathophysiology

Christophe Antczak,^{1,2} Ingrid De Meester,^{1,3} and Brigitte Bauvois^{1*}

Summary

Ectopeptidases are transmembrane proteins present in a wide variety of tissues and cell types. Dysregulated expression of certain ectopeptidases in human malignancies suggests their value as clinical markers. Ectopeptidase interaction with agonistic antibodies or their inhibitors has revealed that these ectoenzymes are able to modulate bioactive peptide responses and to influence growth, apoptosis and differentiation, as well as adhesion and motility, all functions involved in normal and tumoral processes. There is evidence that ectopeptidase-mediated signal transduction frequently involves tyrosine phosphorylation. Combined analyses of gene organization and regulation of ectopeptidases by various physiological factors have provided insights into their structure–function relationships. Understanding the roles of ectopeptidases in pathophysiology may have implications in considering them as therapeutic targets. *BioEssays* 23:251–260, 2001.

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Introduction

Cells communicate with their environment through several kinds of cell surface receptors including cytokine receptors, cell adhesion molecules and ectoenzymes. A distinctive feature of ectoenzymes, represented by nucleotidases, glycohydrolases, phosphorylases, ADAMs (a disintegrin and metalloprotease family), secretases and peptidases, is that they are integral membrane proteins with an active catalytic site exposed to the external surface of the membrane.^(1–3) This article focuses on ectopeptidases. Today, around twenty ectopeptidases have been identified in human cell types and tissues (Fig. 1). They are anchored in the plasma membrane either with the N terminus or the C terminus facing extracellularly or through the glycosyl-phosphatidylinositol moiety (Fig. 1). Ectopeptidases lead a dual existence as membrane-bound and soluble isoforms found in body fluids (Fig. 1). Enzyme release is sometimes associated with pathology as seen in granulomatous inflammation with overproduction of serum angiotensin-

converting enzyme (ACE), or during the course of hepatitis C characterized by enhanced levels of serum γ -glutamyl transpeptidase (γ -GT). The mechanisms for regulating the shedding of membrane ectopeptidases are unknown except for ACE which is released by a member of the secretase family.⁽²⁾ Interestingly, certain surface molecules initially identified by immunologists and hematologists as cluster of differentiation (CD) antigens are identical to well-studied ectopeptidases i.e. aminopeptidase N (APN), dipeptidyl peptidase IV (DPPIV), neutral endopeptidase (NEP) and ACE (Fig. 1). Based on their aminoacid sequences, ectopeptidases belong to different families and clans of proteases indicating their different origin. At present, there is no evidence for extended sequence homology between all members of ectopeptidases that could explain their developmental regulation and their functional similarity. The structure and enzymatic action of most ectopeptidases have been the subject of recent reviews^(4–12) and will not be detailed here. Although almost all ectopeptidases are widely distributed, their levels of expression vary considerably during the fetal/postnatal development stages^(4–13) and in inflammatory reactions.⁽¹³⁾ Several investigations have reported that physiological stimuli regulate ectopeptidase expression and will be summarized here. An essential question for clinicians and scientists is whether ectopeptidases critically contribute to the disease process. We will describe here salient advances in the molecular genetics of ectopeptidases, and what is currently known of their biological activities.

Expression of ectopeptidases in disease

There are many reports of the dysregulated expression of ectopeptidases in human leukocyte malignancies and they have been frequently used as diagnostic markers (Table 1; Fig. 2). NEP, DPPIV and γ -GT are overexpressed in several forms of B leukemias and T/B lymphomas.^(4,6,14,15) APN and γ -GT are overexpressed in acute and chronic myeloid leukemias.^(16–18) In contrast, the loss of DPPIV and γ -GT from the surface of lymphocytes is associated with acute and chronic T lymphocytic leukemic diseases.^(19,20) Ectopeptidases also appear to be dysregulated in putative autoimmune diseases such as multiple sclerosis (21, 22) and rheumatoid arthritis^(23,24) as well as sarcoidosis⁽⁷⁾ and HIV infection^(6,25) (Table 1).

Outside of the hematopoietic system, the expression pattern of ectopeptidases in solid tumor malignancies has been extensively reviewed by Nanus and collaborators⁽²⁶⁾ and

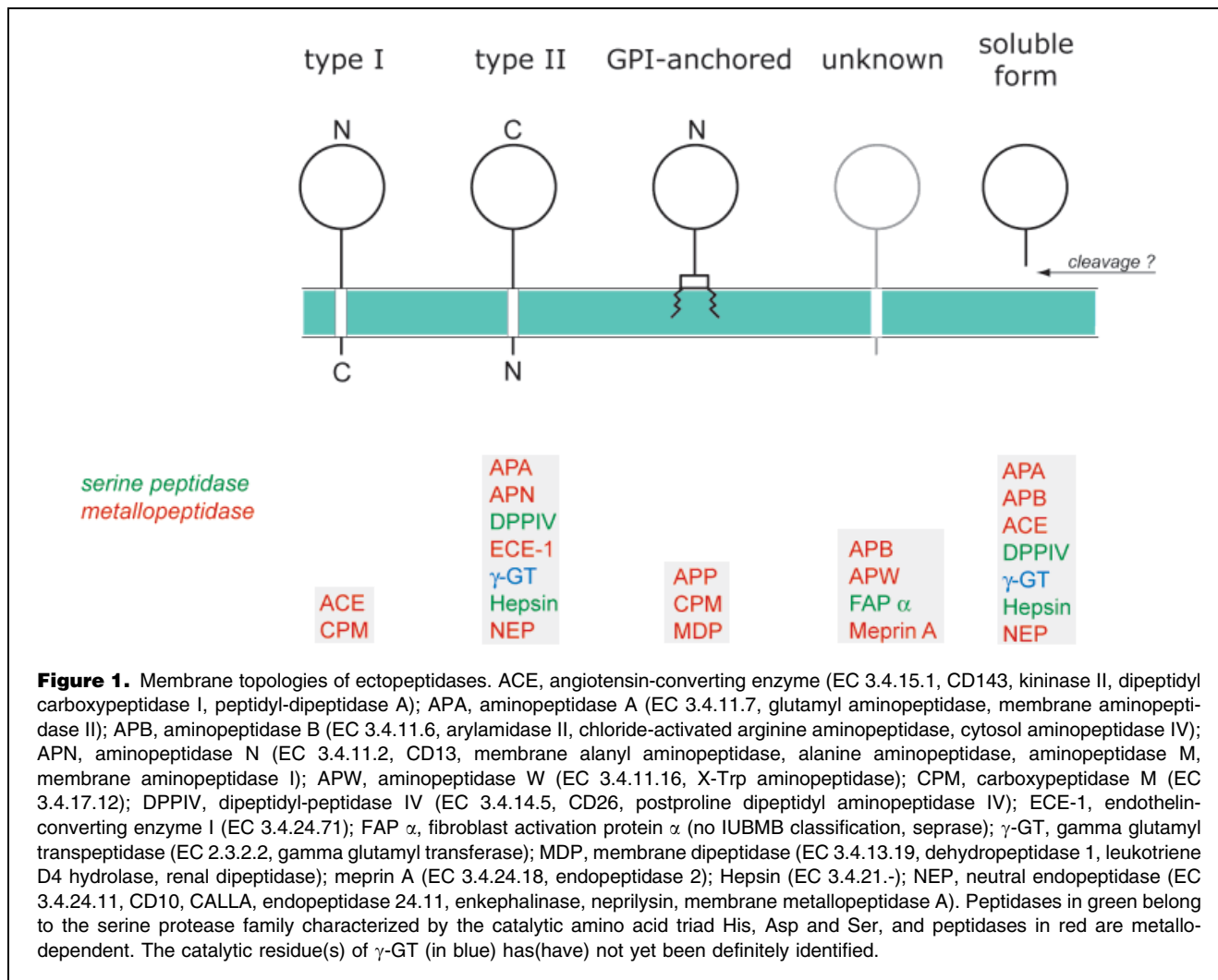
¹Institut Curie, INSERM 365, Paris, France.

²Institute Curie, CNRS UMR 176, Paris, France.

³Department of Pharmaceutical Sciences, University of Antwerp, Belgium.

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*Correspondence to: Brigitte Bauvois, Unité 365 INSERM, Institut Curie, 26, rue d'Ulm, 75231 Paris cedex 05, France; E-mail: brigitte.bauvois@curie.fr



others^(11,12,27–29) (Table 1). Often, loss of expression or conversely overexpression of ectopeptidases in tumors has been linked to tumor initiation, invasion and metastasis. The only example of a causal relationship between ectopeptidase expression and pathology, however, is seen in human melanocytes where the loss of DPPIV is invariably associated with malignant transformation.⁽²⁹⁾

Regulation of ectopeptidases by physiological stimuli

Ectopeptidases can be induced or enhanced by treatment with physiological agents and such regulation may be transcriptional, translational or relate to translocation at the cell surface (Table 2). Granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor- α (TNF- α), substance P and formyl-methionyl-leucyl-phenylalanine (f-MLP) increase the expression of NEP at the cell surface of neutrophils.⁽¹³⁾ While interferon- γ (IFN- γ) induces APN and

DPPIV in epithelial cells,^(5,30) all three classes of IFNs are capable of upregulating DPPIV in B-CLL.⁽³¹⁾ Some interleukins enhance APN, DPPIV and γ -GT in various cell types including epithelial cells and leukocytes.^(5,32–34) Glucocorticoids stimulate NEP and ACE levels in epithelial and myeloid cell types.^(7,13)

Molecular genetics of ectopeptidases

Gene loci and gene organizations

Several ectopeptidases have been cloned, and the identification of *cis* response elements within their genes may help in the elucidation of ectopeptidase regulation. Studies on the transcription factors regulating ectopeptidase expression and their eventual modifications in malignant cells may provide information essential to understanding their roles.

Ectopeptidases listed in Table 3 are localized at distinct chromosomal loci and their promoters share no close

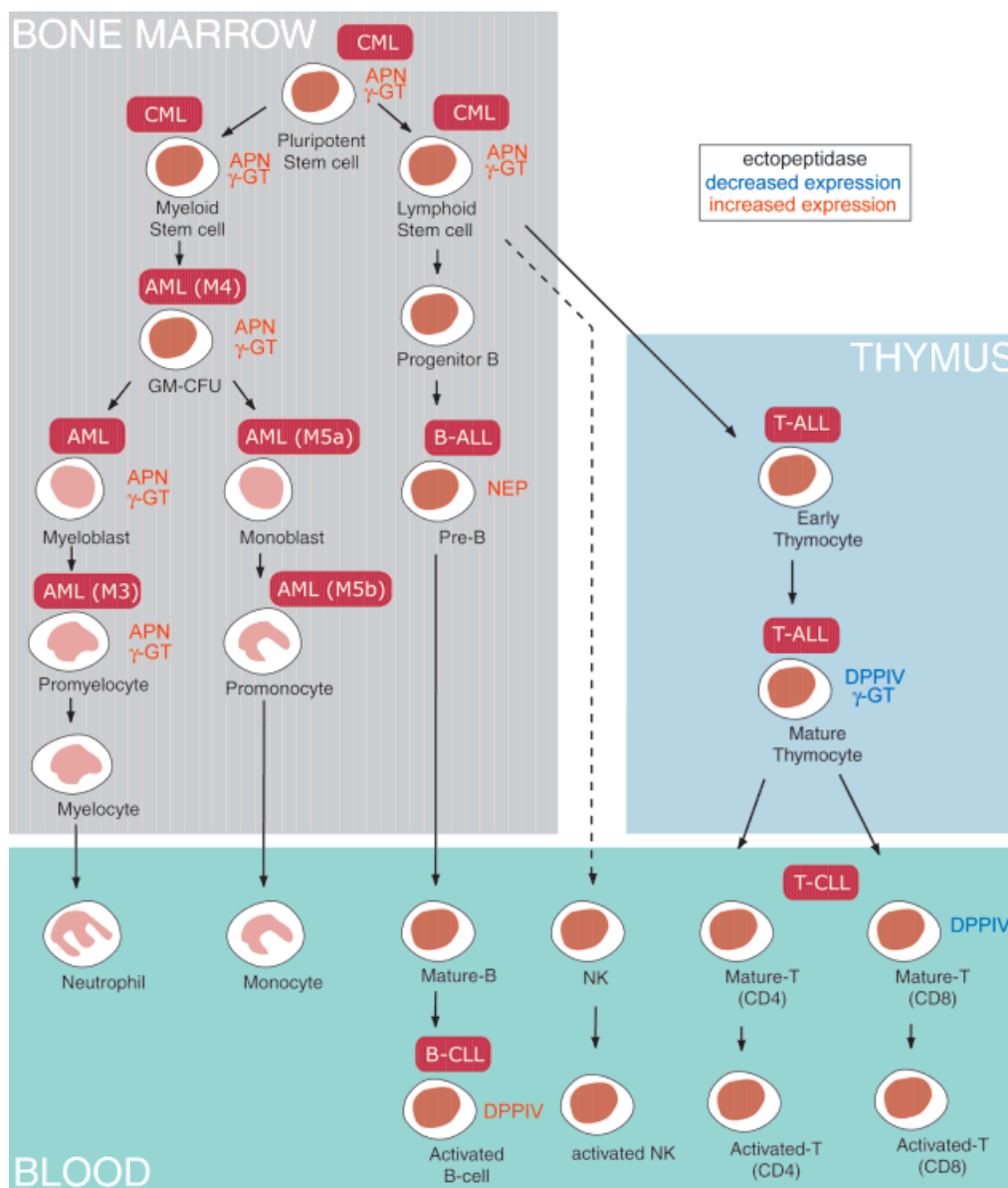


Figure 2. The cell lineages of the hematopoietic system and neoplastic transformation. Mature cells from the various hematopoietic lineages (lymphoid and myeloid) develop from a common pluripotent stem cell through lineage-committed intermediates. The numbers and types of committed progenitor cells have been simplified. The location of hematological diseases (in circles) in this scheme is indicative and does not reflect disease complexity. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; GM-CFU, granulocyte-macrophage colony-forming unit. Ectopeptidases in neoplastic cells are mentioned and their dysregulation is indicated in red in case of overexpression, and in blue for loss of expression.

homology. For NEP, APN and ACE, two distinct promoters have been characterized for each gene.^(4,7,16) These promoters drive different expression patterns in different cell types.⁽⁷⁾ The type I promoter of NEP contains several consensus sequences for members of the *Ets* family of transcription factors (*PU.1* and *PEA3*) and the type II promoter is

characterized by the presence of putative *Sp1*-binding sites and one retinoblastoma control element (*RCE*) (4). Myb and Ets-2 specifically cooperate in the transactivation of the APN gene in myeloid and activated T cells.^(16,17) A mutation in *Ets-1*-binding domain of the APN gene has been reported in one case of acute T lymphocytic leukemia^(16,17) whereas muta-

Table 1. Aberrant expression of ectopeptidases in disease

Pathology	Ectopeptidase involved	Dysregulation
<i>Leukemias, lymphomas</i>		
B-ALL	NEP	↗
B-CLL	DPPIV	↗
B, T lymphomas	NEP, DPPIV, γ -GT	↗
AML, CML	APN, γ -GT	↗
T-ALL, T-CLL	DPPIV, γ -GT	↘
<i>Putative immune diseases</i>		
multiple sclerosis	NEP, APN, DPPIV	↗
arthritis	APN, DPPIV	↗
<i>Sarcoidosis</i>	ACE	↗
<i>HIV infection</i>	NEP DPPIV	↘
<i>Solid tumors</i>		
gastrointestinal cancers	APN	↘
genitourinary cancers	APA, APN Hepsin, FAP- α	↘ ↗
lung cancer	DPPIV	↗
liver cancer	NEP, γ -GT, Hepsin	↗
melanoma	NEP, DPPIV, FAP- α Seprase	↘ ↗
thyroid cancer	DPPIV	↗

All, acute lymphoid leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia. ↗, overexpression of the peptidase; ↘, loss of expression.

tions in other parts of the upstream promoter have been detected in one case of chronic myeloid leukemia and two cases of non-Hodgkin's lymphoma.⁽¹⁷⁾ Whether these mutations contribute to the malignant transformation however remains to be established. The somatic ACE promoter, which contains several potential *Sp1*-binding sites, is active in several cell types including monocytes whereas the germinal ACE promoter is active in male germinal cells.⁽⁷⁾ Importantly, an insertion–deletion polymorphism of the ACE gene is associated with differences in the levels of ACE in plasma.⁽⁷⁾ Deletion induces high expression of ACE reported to represent a risk factor of cardiovascular diseases.⁽⁷⁾

The human DPPIV gene contains several potential binding sites for transcription factors (31, 35) (Table 3). Cotransfection of the murine 3T3 fibroblast cell line with *HNF-1 α* and DPPIV promoter constructs results in an increase of DPPIV transcription.⁽³⁶⁾ Interferons (IFNs) type I (α , β) and II (γ) and retinoic acid upregulate the expression of the DPPIV gene in chronic B lymphocytic leukemia at least through activation of the *GAS* (interferon- γ activated sequence) motif by the transcription factor *Stat1 α* .⁽³¹⁾ Similar to the gene encoding DPPIV, the fibroblast activation protein α (FAP- α) gene locus is located on human chromosome 2 in q23.^(12,37) Human γ -GT is a multigenic family composed of at least seven genes⁽³⁸⁾ and five of them have been identified on the chromosome 22 proximal to

the chronic myeloid leukemia break point in *bcr* (break point cluster region gene).⁽³⁹⁾ The chromosomal loci of other ectopeptidases have been defined (Table 3).

Deletion of ectopeptidase genes in mice

Although caution should be exercised in extrapolating results of mice models to humans, targeted mutations of the genes encoding the few ectopeptidases studied demonstrate their involvement in a number of biological events (Table 3). Deletion of NEP causes a widespread basal plasma extravasation in postcapillary venules related to increased availabilities of substance P and bradykinin,⁽¹²⁾ enhanced endotoxin shock-lethality⁽⁴⁰⁾ and uncontrolled inflammation.⁽⁴¹⁾ Mice lacking ACE have reduced blood pressure, impaired male fertility and kidney defects characterized by arterial thickening and infiltration of inflammatory cells.^(42,43) γ -GT-deficient mice are sexually immature, develop cataracts, express an altered glutathione metabolism⁽⁴⁴⁾ and exhibit impaired T cell number and function.⁽⁴⁵⁾ Interestingly, humans that have γ -GT deficiency exhibit glutathionemia and glutathionuria as in γ -GT-deficient mice but the stigmata are less severe since γ -GT is a multicopy gene in humans compared with the single copy in mice.⁽⁴⁴⁾ Among the substrates of DPPIV, glucagon-like peptide 1 (GPL-1) is important for glucose regulation and in its intact form enhances glucose-stimulated insulin secretion. Targeted inactivation of the DPPIV gene yields healthy mice that have increased levels of GPL-1 and insulin⁽⁴⁶⁾ indicating that DPPIV participates in blood glucose regulation by partially controlling the activity of GPL-1. Finally, FAP- α -deficient mice have no apparent anatomical or developmental abnormalities⁽⁴⁷⁾ suggesting a compensatory regulation by other proteases overlapping FAP- α function.

Biological activities of ectopeptidases

Ectopeptidase functions can be divided into two types, those that require the enzyme activity and those that do not.

Processing of bioactive peptides

The processing of bioactive peptides (cytokines, neuropeptides and hormones) is obviously dependent on the catalytic activity of ectopeptidases. Most ectopeptidases have more than one potential substrate and a given ectopeptidase may be expressed by different cell types, suggesting that each of them contributes to the post-translational modification of a variety of peptides.^(4–10)

By activating, inactivating or changing the receptor specificity of these peptides, ectopeptidases influence proliferation, differentiation, migration and vascular permeability.^(4–7) One well-known system concerns the role of ACE in cardiovascular homeostasis through its proteolysis of the vasoconstrictors angiotensin I and bradykinin.⁽⁷⁾ Some chemotactic peptides are substrates of leukocyte ectopeptidases which therefore

Table 2. Regulation of ectopeptidase expression by physiological stimuli

Stimulus	Ectopeptidase	Cell type	Regulation	
			mRNA	Protein
<i>IFNs</i> α , β , γ	DPPIV	B-CLL	+	+
<i>IFN-γ</i>	APN, DPPIV	epithelial	+	
<i>GMF-CSF</i> , <i>TNF-α</i>	NEP	neutrophil	+	
<i>Substance P</i> , <i>f-MLP</i>	NEP	neutrophil	+	
<i>Glucocorticoids</i>	ACE	myeloid	+	
	NEP	epithelial	+	
<i>IL-2</i>	APN	epithelial	+	+
<i>IL-4</i> + <i>IL-13</i>	APN	epithelial	+	+
<i>IL-12</i>	DPPIV	T, activated NK	+	
<i>IL-15</i>	DPPIV	activated NK	+	
	γ -GT	activated T	+	

f-MLP, N-formyl-methionyl-L-phenylalanine; GM-CSF, growth factor colony-stimulating factor; IFN, interferon; NK, natural killer; TNF, tumor necrosis factor. +, positive regulation demonstrated on mRNA or protein levels.

influence cell chemotaxis. For example, inhibition of NEP and APN enhance neutrophil chemotaxis in response to formyl methionyl leucine phenylalanine (f-MLP) and substance P by preserving the integrity of these inflammatory peptides.⁽¹³⁾ In contrast, DPPIV can alter leukocyte chemotaxis by processing chemokines such as RANTES, SDF-1 α (stromal cell derived factor), eotaxin and MDC (monocyte derived chemokine).⁽⁶⁾ It

seems likely that many physiologically important substrates remain to be defined.

Cell proliferation, apoptosis, differentiation and secretion

Ligation of ectopeptidases with agonistic antibodies (Abs) or exogenous enzyme inhibitors (natural or synthetic) has

Table 3. Molecular genetics of ectopeptidases

Ectopeptidase	Gene	Chromosome	Promoter	Transcriptional elements	Phenotype of $-/-$ mice
NEP	1	3q21-27	2	Ets Sp 1 RCE	<i>hypersensitivity to endotoxin shock</i> <i>increased inflammation</i>
APN	1	15q25-26	2	Ets Myb	—
ACE	1	17q23	2	<i>Sp1</i>	<i>reduced blood pressure</i> <i>kidney dysfunction</i>
DPPIV	1	2q24.3	1	AP1/2 ETF HNF NFKB <i>Sp1</i> Stat1	<i>enhanced insulin secretion</i>
FAP- α	1	2q23	1	—	<i>no apparent alterations</i>
γ -GT	7	γ -GT1: 22q11.1-11.2 γ -GT2: 22q11.12	—	—	<i>defect in GSH metabolism and immune suppression</i>
APA	1	4q25	—	—	—
APB	1	1q32.1-32.2	—	—	—
CPM	1	12q15	—	—	—
ECE-1	1	1q36.1	—	—	—
Hepsin	1	19q11-13.2	—	—	—
MDP	1	16q24.3	—	—	—
Meprin A	1	18q12.2-12.3	—	—	—

ETF, epidermal growth factor receptor specific transcription factor; GSH, glutathione; HNF-1, hepatocyte nuclear factor-1; RCE, retinoblastoma control element. Chromosomal location is published in MEROPS database (<http://www.merops.co.uk>).

revealed additional functions. Most data derive from circulating leukocytes, epithelial cells and established cell lines.

Several studies have indicated that interaction of ectopeptidases with their inhibitors can alter cell proliferation. Inhibition of DPPIV, APN and γ -GT led to the suppression of growth of leukocyte subpopulations (T, myelomonocytic and cord blood CD34⁺).^(5,48–54) Growth arrest by inhibitors of ectopeptidases correlates with altered production of proinflammatory cytokines, as seen for DPPIV (release of TGF- β 1 and suppression of IL-2, IL-10, IL-12 and IFN- γ)^(6,49) and ACE (release of TNF- α , IL-1, IL-12 and tissue factor, the initiator of blood coagulation).^(56–58)

The inhibitor-mediated cell growth arrest can be associated with an induction of cell maturation, as seen with the inhibitors of NEP, APN and ACE, which accelerate the maturation of clonogenic GM-CFU (granulocyte-macrophage colony-forming unit) cells from human immature derived-bone marrow cells.^(59–61) Similarly, treatment of human myeloid cell lines or blood monocytes with inhibitors to APN or γ -GT induces phenotypic changes characteristic of macrophage maturation.^(17,52–54)

Recent observations point to the involvement of ectopeptidases in the process of programmed cell death. Inhibitors of APN and γ -GT induce apoptosis of T and myeloid cell lines.^(51,55,62) Unlike in HIV-infected jurkat T cells in which transfection of DPPIV cDNA protects them from apoptosis,⁽⁶⁾ engagement of DPPIV in a hepatocarcinoma cell line through DPPIV Ab, delivers a potent apoptotic signal⁽⁶⁴⁾ suggesting that DPPIV exerts opposite functions in different cell contexts.

Cell invasion

Ectopeptidases might be implicated in cell invasion by their abilities to adhere to and degrade the extracellular matrix. DPPIV of epithelial, fibroblast and T cells binds to the macromolecules fibronectin and collagen and this binding can be blocked by antibodies to DPPIV.^(6,48) Activated T cells expressing high levels of DPPIV preferentially migrate through a monolayer of endothelial cells on collagen gels⁽⁶⁴⁾ while APN mediates the migration of human metastatic cell lines through matrigel.⁽⁵⁾ FAP- α is directly involved in the degradation of collagen which is abundant in inflamed tissues⁽¹²⁾ whereas CD13 participates indirectly in this process by inducing the secretion of type IV collagenase by epithelial cells.⁽⁵⁾

Ectopeptidases as receptors

APN is reported to act as a receptor for the coronavirus 229E, which infects epithelial cells of the respiratory tract, and to mediate human cytomegalovirus infection (responsible for herpes) by enhancing its binding to the surface of infected cells.⁽⁶⁵⁾ DPPIV has been demonstrated to bind adenosine deaminase (ADA), lack of which causes severe impairment of cellular and humoral immunity.⁽⁶⁾

Ectopeptidases and signal transduction pathways

One feature common to ectopeptidases is that they possess a short intracytoplasmic domain (less than 20 amino acids) with no obvious motifs.^(4–12) How then might signals be transduced by such receptors? Their connection to functional molecules that may channel activation signals has been investigated. Published observations on ectopeptidase signaling are confined to DPPIV and APN. Although a physical association between DPPIV and CD45 (a protein tyrosine phosphatase exclusively expressed in the hematopoietic system) has been reported, the importance of this association for signaling is still unknown.⁽⁶⁾ A very recent study demonstrated that the interaction between DPPIV and the mannose 6-phosphate receptor that was previously identified as the insulin-like growth factor II receptor, contributes to T cell activation.⁽⁶⁶⁾ In T cells, triggering of DPPIV by Ab is associated with calcium mobilization and activation of cellular proteins involved in TcR/CD3-mediated signal transduction (CD3 zeta chain, Src kinases, ZAP70, MAPKs and PLC).⁽⁶⁾ DPPIV inhibitors activate MAPK-p38 and suppress CD3 Ab-mediated activation by inhibiting MEK1 and ERK1/2.⁽⁴⁹⁾ Related intracellular effectors (calcium increase, the MAP kinases ERK1/2, JNK and p38) appear to be implicated in the activation of T cells through APN.^(5,17)

Although the basis for signal transduction via γ -GT is still unknown, molecular associations between γ -GT and some members of the tetraspan 4 family including CD53, CD81 and CD82, are reported.⁽⁶⁷⁾ It remains to be seen whether γ -GT-mediated events require these molecules. Interestingly, the biochemical pathways initiated by ectopeptidase engagement overlap the signals triggered by integrins and cytokines. For instance, MAP kinases regulate cell proliferation following binding of a cytokine to its receptor or integrin-mediated adhesion^(68,69) (Fig. 3).

At present, the mechanisms of action of inhibitors and Abs is not completely clear. Indeed, with regard to APN and DPPIV, the enzymatic activity, even if it contributes, does not appear essential for signal transduction.^(6,17) Inhibitor binding to the ectopeptidase, as with Ab binding, might induce conformational changes of the enzyme, thus initiating lateral membrane interactions with signaling molecules.

Conclusions

Understanding the roles of ectopeptidases has reached a critical step. They may exert biological activities by molecular properties dependent or independent of their enzymatic activities (Fig. 4). Since ectopeptidases are dysregulated in various diseases (Table 1), they represent a likely target for intervention in pathophysiological situations. For several years now, ACE inhibitors have proved their efficacy in the treatment of hypertension, diabetic nephropathy⁽⁷⁰⁾ and post-transplantation erythrocytosis.⁽⁷¹⁾ As monotherapy, there is evidence

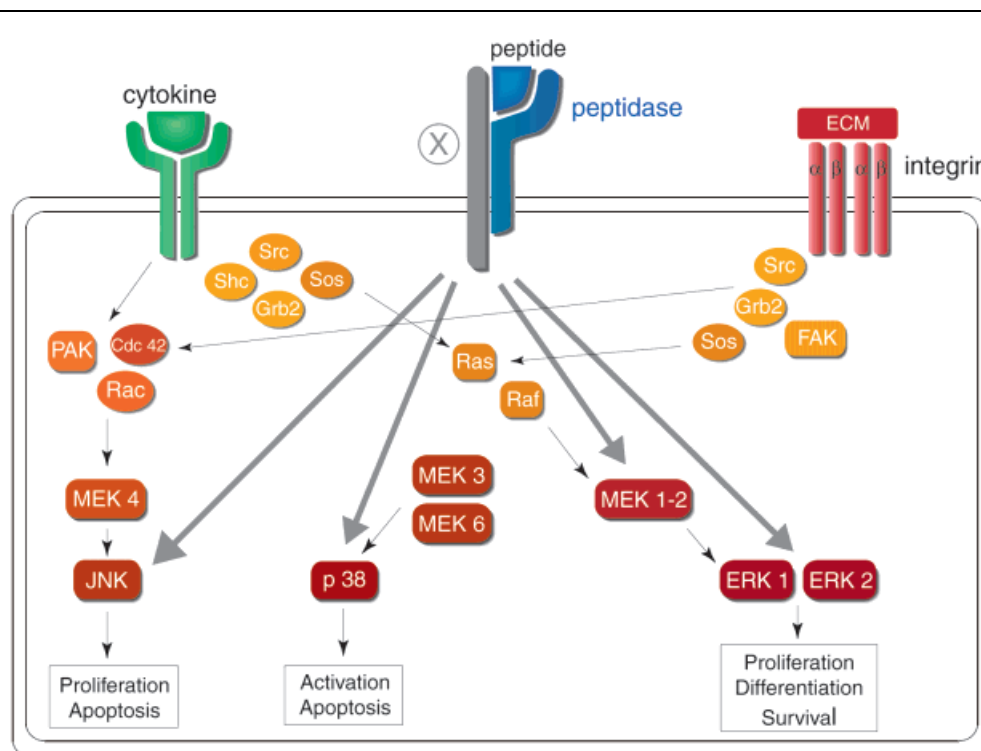


Figure 3. Activation of MAPK pathways downstream of ectopeptidases and convergence with signal transduction pathways of integrins and cytokine receptors. Three subfamilies of MAPKs (mitogen-activated protein kinase) are ERK (extracellular signal regulated protein kinase), JNK (c-Jun N-terminal kinase) and p38. Activated ERKs can translocate to the nucleus and phosphorylate several transcription factors like AP-1. MEK, MAPK/ERK kinases; The Ras superfamily (including Ras and Rho groups) belong to the larger group of GTP-binding proteins or G proteins. Rac and Cdc42 belong to the Rho subfamily. The signal Ras/Raf-MEK-MAPK transduction pathway is activated by almost all growth factors. ECM, extracellular matrix; FAK, focal adhesion kinase; Grb2, growth factor receptor bound SH2/SH3-containing adapter protein; SOS, product of Son of Sevenless gene; Grb2/SOS: guanine nucleotide exchange complex for Ras; PAK, p21-activated kinases; src, sarc; X, unknown signaling-molecule. ERK, p38, JNK and Raf are serine/threonine kinases; MEK, Src and FAK are tyrosine kinases.

that NEP inhibitors have beneficial hemodynamic effects in patients with heart failure.⁽⁷⁰⁾ Another active area of pharmaceutical research concerns the use of DPPIV inhibitors in type 2 diabetes. We expect that future clinical investigations using highly specific inhibitors may reveal the prominent functions of the targeted ectopeptidases. Limitations to the use of inhibitors in vivo, however, include the wide distribution of these enzymes, the toxicity and/or the lack of specificity observed for some of their inhibitors. Many patients taking ACE inhibitors experience a troublesome dry cough that appears to result from increased levels of bradykinin and/or substance P.⁽⁷⁰⁾ There is thus a need to design of an inhibitor-based product that displays no toxicity, high specificity and capability of reaching the target enzyme where needed. One approach could be to synthesize inhibitors linked to vehicles such as antibodies that react with target cell surface antigens. The

principle has already been used to target toxins to solid tumors and malignant leukocytes.^(72,73)

Two other areas of future research deserve mention. One concerns the definition of the in vivo substrate(s) of a given ectopeptidase. Indeed, although peptidase recognition and cleavage patterns have been characterized in vitro using recombinant peptides, not all physiological substrates have yet been identified. Second, the role(s) of soluble forms of ectopeptidases in biological fluids are still poorly understood. Although the involvement of soluble γ -GT in the transport of glutathione is obvious, the specific function(s) of other soluble ectopeptidases is (are) still unknown. By solving these questions, we shall enhance understanding of the assembly of the ectopeptidase network controlling communication of cells with their environment, and may see the emergence of new drugs with highly selective properties.

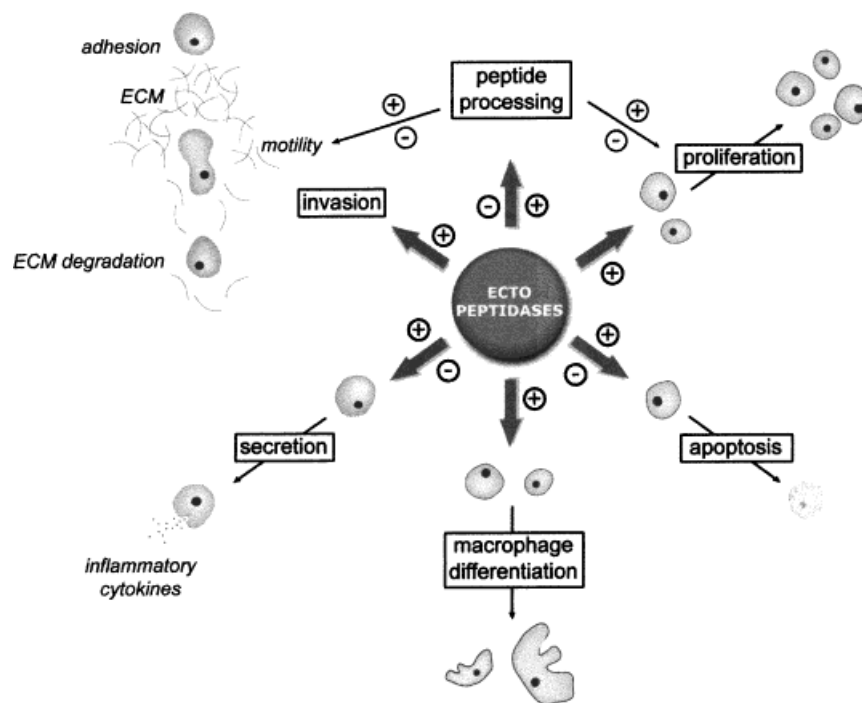


Figure 4. Biological effects of ectopeptidases. The actions of ectopeptidases *in vitro* are diverse and range from the regulation of cell proliferation to cell invasion. ECM, extracellular matrix. Their potential *in vivo* actions deriving from the use of knock-out mice are discussed in the text. +, positive effect; -, negative effect.

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