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Cell-surface proteolysis, growth factor activation and intercellular communication in the progression of melanoma

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Abstract

Normal skin architecture and melanocyte function is maintained by a dynamic interplay between the melanocytes themselves, the epithelial cells between which they are interspersed, and their microenvironment. The microenvironment consists of the extracellular matrix, fibroblasts, migratory immune cells, and neural elements supported by a vascular network, all within a milieu of cytokines, growth factors, and bioactive peptides as well as proteolytic enzymes. Cells interact with the microenvironment via complex autocrine and paracrine mechanisms. Proteolytic enzymes in melanoma may activate or release growth factors from the microenvironment or act directly on the microenvironment itself, thereby facilitating angiogenesis or tumor cell migration. This review summarizes recent findings regarding the expression, structure and function of proteolytic enzymes at or near the cell surface in cell–cell and cell–stroma interactions during melanoma progression. Cell-surface (membrane) peptidases are a multi-functional group of ectoenzymes that have been implicated in the control of growth and differentiation of many cellular systems. The potential, but yet speculative, role of other membrane-bound molecules, such as multifunctional surface proteins with adhesion and protease activity (ADAM gene family) or the ephrin/Eph receptor protein kinases in the pathogenesis of melanoma are discussed. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Melanoma; Proteolysis; Microenvironment; Stroma; Cell-surface peptidases; Ephrins; Eph receptors

1. Introduction—or: why is cell-surface proteolysis important in tumorigenesis?

Normal skin homeostasis is maintained by dynamic interactions between the melanocytes and their microenvironment, such as keratinocytes, fibroblasts, endothelial and immunocompetent cells, and the extracellular matrix. Melanocytes adhere to keratinocytes, whereas communication between melanocytes and fibroblasts or endothelial cells occur through soluble factors. During the transformation and progression of melanocytes and melanoma cells, there are reciprocal interactions between the neoplastic cells and adjacent normal skin cells, such as dermal and epithelial cells (see [1,2] for review).

Cancer and melanoma research over the past decades has been largely focused on events occurring within the boundaries of the plasma membrane of the malignant cell. The dominant paradigm, wherein multiple genetic lesions, e.g. of the cyclinD/cdk4-p16INK4A-pRb-pathway [3,4], provide both the impetus for and the possible Achilles heel of cancer, which in return can be targeted for gene therapy [5], is not sufficient to understand melanoma as a disease process. Furthermore, some of the genetic lesions frequently encountered in other solid tumors, e.g. alterations of the p53 tumor suppressor gene product, are apparently not of relevance in the evolution of melanoma [6,7]. Considering that 2% of the gene products of organisms, whose genome has been sequenced are proteases [8], many exciting discoveries about the functions of these molecules in physiological and neoplastic processes can be expected in the future. In the following review, we will use selected examples to illustrate the influence of cell-surface proteolysis and the resulting alteration of the pericellular microenvironment for the evolution of melanoma.

2. From slave to master: selected players in maintaining normal skin architecture

The basic properties of cellular behavior that define function are growth, morphology, polarity, adhesion, migration, and expression of tissue-specific proteins [9]. These properties constitute the cell phenotype, which is conferred by interaction between the expression of spe-
Melanocytic tumors: Maturation, Differentiation, Transformation and Progression

Fig. 1. Biological events leading to melanoma development and progression. The model, developed by Clark et al. [19], implies that melanoma commonly develops and progresses in a sequence of steps from nevus lesions, which can be histologically identified in approximately 35% of cases. However, melanoma may also develop directly from normal cells. The role of melanoblasts (immature melanocytes) in melanogenesis remains poorly defined. The progression from normal melanocyte to nevus may be initiated by loss of contact between melanocytes and keratinocytes, i.e. the melanocytes escape from keratinocyte (KC) control. Genetic changes, which are currently not defined, are expected at the transition from common acquired (benign) nevus to dysplastic nevus/RGP/in situ melanoma (left vertical arrow), allowing cells to persist. Additional genetic changes are expected in the progression from RGP/in situ melanoma to VGP (right vertical arrow). At the VGP (tumorigenic) step, increased growth, invasion and stromal ‘landscaping’ by proteolysis occurs.

Specific genes and the cells’ responses to ECM, to neighbouring cells and to soluble effectors, such as growth factors and cytokines [5,10].

Normal melanocytes are tightly controlled by keratinocytes (Table 1). Keratinocytes, the ‘masters’, dictate when the melanocytes, the ‘slaves’, can grow and what cell-surface molecules are expressed [11,12]. The keratinocytes need cell-cell contact to establish this control, which is mediated by E-cadherin. E-cadherin is found on normal melanocytes and to a lesser degree on nevi and little on melanomas (Fig. 2) [13]. The loss of E-cadherin expression has significant biological consequences in melanocytic cells. Melanoma cells have escaped from keratinocyte control by shutting off expression of E-cadherin and activating N-cadherin [14]. They can now leave the epidermis, invade the dermis and closely adhere to and communicate with fibroblasts, endothelial cells, and other stromal cells and components. The ‘run-away’ slave has become a powerful ‘master’, accepting growth factors from keratinocytes; it now directs the presence and functions of fibroblasts, endothelial and inflammatory cells in its microenvironment. The melanoma cells tell the fibroblasts to produce a scaffolding with matrix proteins, and to release growth factors, which melanomas cannot synthesize on their own, but which increase their growth, survival and invasive capacity [15]. The symbiosis has been reversed and the malignant melanoma cells are in the driver’s seat.

The escape of melanoma cells from the epidermis can be experimentally reversed. Forced reexpression of E-cadherin in melanoma cells leads to growth retardation, inhibition of invasion and induction of apoptotic death in three-dimensional skin reconstitutions, and decreased tumorigenicity in mice [14]. Thus, E-cadherin may act as an invasion suppressor in the melanoma system. Melanoma cells, even the most aggressive metastatic ones, can again come under the control of keratinocytes, if the expression of E-cadherin is re-established by gene transfer [16]. The N-cadherin gene is then downregulated and the melanoma cells no longer establish gap junctions with fibroblasts [14]. The keratinocytes are again in the driver’s seat: They can adhere to the E-cadherin expressing melanoma cells and dictate whether these grow or not [16]. Within a few days, all melanoma cell surface molecules associated with growth, invasion and metastasis are shut off. Important markers are the β3 integrin subunit that allows biologically early melanoma cells to invade into the dermis [17,18], and the cell-cell adhesion marker Mel-CAM/MUC18 [12,18]. We do not know the mechanisms, by which keratinocytes can transmit their signals, but these signal are strong enough to force the melanoma cells back into a subservient position.

3. Melanoma development is a multi-step process

Based on clinical and histopathological features, five steps of melanoma progression have been proposed (Fig. 1) [15,19]: common acquired and congenital nevi with structurally normal melanocytes, dysplastic nevi with structural and architectural atypia, early radial growth phase (RGP) primary melanoma, advanced vertical growth phase (VGP) primary melanoma with competence for metastasis, and metastatic melanoma. Despite a wealth of research resources (tissues, cell lines, and antibodies), the genetic and biochemical alterations responsible for the development and stepwise progression of melanoma still remain enigmatic. Cytogenetic analyses have failed to identify consistent gene deletions, mutations, translocations, or amplifications in sporadic cases [1,2].
Fig. 2 summarizes selected genetic and biological events leading to melanoma development and progression. The dynamic progression from a resting melanocyte to a common acquired nevus is very common and does not appear to accompany genetic changes. Nevus cells isolated from common acquired nevi have a finite life span and generally do not carry cytogenetic abnormalities [20–22].

We postulate that melanocytes progress to a nevus by escaping from the normal contact-mediated controls of keratinocytes. Keratinocytes are the dominant cellular partner of melanocytes in the epidermis and control the growth, morphology, and antigenic phenotype of melanocytes [11,23] by establishing direct contact through the cell-cell adhesion receptor E-cadherin. This contact, in turn, facilitates formation of gap junctions through connexin 43 [14]. It remains unclear, whether signals for phenotypic control over melanocytes are relayed through E-cadherin, gap junctions or other accessory mechanisms. Nevertheless, E-cadherin down-regulation coincides with melanoma progression. Reduced E-cadherin expression can be observed early in the nevus stage and the majority of melanomas are E-cadherin negative [13].

In contrast, expression of N-cadherin is upregulated in nevi and melanomas. Such a shift in cadherin profile confers new adhesive properties to the cells. Acquisition of N-cadherin may allow gap junctional communication of nevus and melanoma cells with N-cadherin-expressing fibroblasts and endothelial cells [15]. Genetic changes are anticipated when dysplastic nevi develop, but the nature of these changes is currently unknown. It is possible that mechanisms leading to persistence and proliferation of dysplastic nevi rest in the dysfunction of the physiological cascade of apoptosis. Progression from dysplasia to RGP primary melanoma is gradual and spontaneous, and may not require additional molecular changes [15]. The transition from RGP to VGP is a biologically and clinically critical step, accompanying additional genetic abnormalities. However, the specifics are largely unknown. In sections of lesions and in cultured cells, we have described a variety of changes at the biological level, which explain RGP to VGP progression [24].

Unlike RGP melanomas, VGP cells are metastasis-competent [25] and easily adapted to growth in culture. In addition, VGP cells are less dependent on exogenous growth factors [26] and have growth characteristics similar to metastatic cells, such as anchorage-independent growth in soft agar and tumorigenesis in immunodeficient mice. VGP primary melanomas display numerous cytogenetic abnormalities, suggesting considerable genomic instability. No major additional genetic changes may be required for further progression to metastatic dissemination since most VGP melanomas can be readily adapted to a metastatic phenotype through selection in growth factor-free medium or by induction of invasion through artificial basement membranes [27]. This suggests that micro-environmental factors, such as cell–matrix and cell–cell signaling are critical for the metastatic phenotype.

4. Gatekeepers, caretakers and landscapers

The prevailing paradigm for the development of cancer is a multi-step process, during which a cell acquires multiple genetic mutations [5,9,28]. The central question that has dominated the literature in the past years is: how many and what genetic changes are necessary for a cell to become malignant [5,9]? In a step towards functionally categorizing these genetic changes, Kinzler and Vogelstein have classified the genes involved, as those that monitor growth by suppressing proliferation, inducing apoptosis or promoting differentiation (‘gatekeepers’). These are assisted by genes that indirectly suppress neoplasia by ensuring the fidelity of the DNA code through effective repair of DNA damage or by

**Fig. 2. Dynamic changes in expression of adhesion receptors, ECM proteins and proteolytic surface enzymes in melanoma progression.** Decreased expression (downward arrow) is seen for some cadherins, CAMs, integrins, and cell-surface peptidases. A strong increase (upward arrow) is seen for a variety of adhesion-related molecules and cell-surface peptidases, first in nevi, then in VGP primary melanomas.
regulating genomic stability (‘caretakers’) [29]. Recently they have also recognized enabling genes (‘landscapers’) [30], which might affect non-target cells by modulating the microenvironment, in which tumor cells grow, perhaps by direct/indirect regulation of extracellular matrix proteins, cell-surface markers, adhesion proteins, or secreted growth factors [31]. Others refer to the aforementioned by the well-accepted term of microenvironmental ‘effectors’ [5].

Malignant tumors are complex tissues, composed of many cell types, such as fibroblasts, endothelial and inflammatory cells, and cannot exist in isolation [9]. Thus, normal cells within the neoplastic tissue are not idle bystanders, but active participants that shape the frequency and features of malignant tumors. Hence, the multi-step genetic modification theory often fails to acknowledge the significance of such forces in the development of neoplasia [5]. Biological events are now beginning to be understood in terms of specific proteolytic proteins affecting cell–cell contacts, cell adhesion and their dynamic reciprocal interaction.

5. Stroma and the pericellular microenvironment

The pericellular microenvironment (‘stroma’) of the normal melanocyte and its malignant counterpart, the melanoma cell, is remarkably complex and consists of cellular, molecular and mechanical components. The insoluble extracellular matrix (ECM) [32] is composed of proteins, glycoproteins, proteoglycans, and glycosaminoglycans in a complex arrangement that provides structure, generates biological signals, stores factors that generate biological signals, and exerts mechanical influences on the epidermis, including the melanocyte. Cells that influence a melanoma cell or its normal progenitor, the melanocyte, include keratinocytes, fibroblasts, adipose cells, endothelial and resident immune cells (in skin: Langerhans cells), each of which represents a heterogeneous population of cellular phenotypes [1,2]. In addition, the stroma has temporal and spatial complexity: it changes with time and tumor progression and is tissue-type specific. The specific molecules that are responsible for tumor-induced changes in the microenvironment and the reciprocal modifications of the tumor by the microenvironment are starting to be known, as are the intracellular pathways that result from these influences.

For example, Mel-CAM/MUC18 is an adhesion receptor that is involved in cell–cell interactions. Its expression is upregulated during melanoma development in a step-wise fashion and coincides with the separation of nevus cells from keratinocytes (Fig. 2) [12]. Mel-CAM binds to a currently unidentified ligand [18], and may play a major role in metastasis by mediating not only melanoma cell–cell interactions, but also melanoma-endothelial cell adhesion. Mel-CAM appears to act in concert with αvβ3, the vitronectin receptor, in promoting metastasis. As the cells progress from RGP to VGP, expression of αvβ3, α2β1, α3β1, α4β1, ICAM-1, and GD2 ganglioside is increased. The most notable marker is the beta3 subunit of αvβ3 integrin, which appears to be the most specific melanoma-associated marker distinguishing RGP from VGP melanomas (Fig. 2) [33]. It is also a prime candidate for prognostic studies [34].

6. ECM and cell-surface proteolysis regulating cellular ecology

The cell-surface and the pericellular space are a dynamic microenvironment. Cell–cell and cell–ECM interactions provide cells with information essential for controlling morphogenesis, cell fate specification, gain or loss of tissue-specific functions, cell migration, tissue repair, and cell death [10,32,35].

During cellular responses to developmental or pathological cues, ECM, cell surface proteins, and receptors are activated or removed by proteolysis [36,37]. Seques-
tration, presentation or activation of growth factors is also regulated by proteolysis [10,38]. One often overlooked aspect of pericellular proteolysis is its potential role in angiogenesis [39], immunity and host defense. Deficient proteolysis leads to disease processes, just as overproduction of proteinases does. Another level of complexity derives from the multiple cell types involved in protease expression within a tumor. In many types of carcinomas, matrix-degrading proteases or cell-surface peptidases are produced not by the epithelial cancer cells but by surrounding stromal and inflammatory cells [10,40].

Over the past decade, cell biology has firmly established in model systems that the complex interactions between epithelial cells and the microenvironment are critical for maintaining a normal, balanced homeostasis [5]. We will now discuss in more detail evidence illustrating the contribution of the microenvironment to normal melanocyte homeostasis. Disrupting this balance by altered cell surface proteolysis can induce aberrant cell proliferation, adhesion, function and migration that might promote malignant behavior of melanocytes.

7. Cell-surface peptidases: hydrolyzing bioactive peptides as a critical component of growth control

Cell-surface peptidases are a group of ubiquitously occurring ectoenzymes with a broad functional, pleiotropic repertoire (Table 2, Fig. 3). They are integral membrane proteins of the plasma membrane, asymmetrically oriented with the catalytic site exposed
| Cell surface peptidase                  | Regulatory function                                                                                   | Known substrates                                                                 | Role in melanoma                                                                 |
|----------------------------------------|-------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| APN (Aminopeptidase N, CD13, EC 3.4.11.2, 140 000-Da glycoprotein) | Reduces cellular responses to peptide hormones. Processes peptides at the intestinal and renal proximal tubular brush borders. Serves as coronavirus receptor. Limits the analgesic effects of opioid peptides. | Opioid peptides, fMLP, enkephalins                                              | Melanocytes: not expressed. Dysplastic nevi: 25%. Melanoma in situ: 44%. Invasive melanoma: 67%. Metastatic melanoma: 100%. Adhesion to extracellular matrix, hydrolyses ECM proteins, activator of type IV collagenase. |
| NEP (Neutral endopeptidase, CD10, EC 3.4.24.11, enkephalinase, neprilysin), 100 000-Da glycoprotein | Reduces cellular responses to peptide hormones. Processes peptides at the intestinal and renal proximal tubular brush borders. Limits the analgesic effects of opioid peptides. | Opioid peptides, fMLP, substance P, bombesin-like peptides, atrial natriuretic factor, endothelin, oxytocin, bradykinin, angiotensins I and II, α-MSH (α-melanocyte-stimulating hormone) | Melanocyte: expressed; downregulated upon UVB-irradiation. Melanoma: all stages 30-40% |
| DPP IV (Dipeptidyl peptidase IV, CD26, EC 3.4.14.5, adenosine deaminase-binding protein), 120 000-Da glycoprotein | Reduce cellular responses to peptide hormones. Processes peptides at the intestinal and renal proximal tubular brush borders. Adhesion molecule: Collagen ECM degradation. | Substance P, casomorphin, kenzin, z chain of fibrin, growth hormone releasing hormone, RANTES (regulated on actiation, normal T-cell expressed and secreted), MCP-1/-2/-3 (monocyte chemotactic protein) | Melanocyte: consistently expressed. Dysplastic nevi: loss of expression. Primary and metastatic melanoma: absent |
Fig. 3. Melanoma-associated cell-surface peptidases. Cleavage site and enzymatic activity (modified from [42]).

at the external surface of the cell [41]. They are widely distributed in human tissues and the physiologic consequences of their activity vary according to their cellular location (reviewed in [42]).

In protein metabolism, their functional importance is well documented, especially in peptide degradation and amino acid scavenging in the brush border membranes of renal and intestinal microvilli: peptidases hydrolyze peptides to facilitate absorption by enterocytes in the intestinal brush-border membrane (in this location 6–8% of the protein is aminopeptidase N [APN]), and recycle amino acids in the brush border of kidney proximal tubule cells [41]. They also perform more subtle physiological tasks. For example, in synaptic membranes, APN and neutral endopeptidase (NEP, enkephalinase) inactivate endorphins and enkephalins [43,44]. They cleave bioactive peptides (Fig. 3), resulting in activation or inactivation, and function as receptors and as molecules participating in adhesion or signal transduction (reviewed in [42] and [45]). Hence, cell-surface peptidases might have a key role in the control of growth and differentiation of many cellular systems by modulating the activity of peptide growth factors and by regulating their access to adjacent cells [41] (Table 2). Whether the enzymatic activity is necessary for all of these different functions remains to be determined [45].

In hematopoiesis, the expression of cell-surface peptidases is a characteristic of several distinct developmental stages. The classification of leukemias and lymphomas is based in part on the expression of cell-surface antigens, which are also present on normal precursor hematopoietic cells. For example, molecules, such as CD10/NEP (common acute lymphoblastic leukemia antigen [CALLA]) and CD13/APN have been used for years in the characterization and typing of leukemia or lymphoma cells [46]. Subsequent analyses of cloned cDNAs identified three of these differentiation antigens, as well-known membrane peptidases with common structural and regulatory features (reviewed in [42]): Aminopeptidase N (APN, CD13, EC 3.4.11.2), neutral endopeptidase (NEP, CD10, CALLA, EC 3.4.24.11, enkephalinase, nephrilysin), and dipeptidyl peptidase IV (DPPIV, CD26, EC 3.4.14.5).

Cell-surface peptidases are also involved in the control of cell growth and differentiation by controlling the access of peptide growth factors to their receptors on the cell membrane [47–49] and in the final steps of collagen degradation in the ECM [50] (Table 2). Therefore, control of bioactive peptides through either activation or inactivation by cell-surface peptides is a critical component of growth control. This idea has also direct implications for the development of neoplasia. Two basic mechanisms of cell-surface peptidase involvement in carcinogenesis can be predicted [46]: (1) loss of function, resulting in an inability of the transformed cell to inactivate a mitogenic peptide or activate an inhibitory peptide; and (2) gain of function, resulting in the activation of a mitogenic peptide or the inactivation of an inhibitory peptide. Consequently, abnormalities in expression pattern and/or catalytic function of cell-surface peptidases result in altered peptide activity, which contributes to neoplastic transformation and/or progression. Data, which implicate specific cell-surface peptidases in the pathogenesis of human cancers (reviewed in [46]), including melanoma, are beginning to emerge. We will now discuss which evidence to date indicates a role for cell-surface peptidases in the development of melanoma.
7.1. Dipeptidyl peptidase IV (DPP IV, CD26, EC 3.4.14.5)

Dipeptidyl peptidase IV (DPPIV) is the best characterized cell-surface peptidase in melanoma. It is a type II membrane glycoprotein with multiple properties, including serine protease activity and the ability to bind ECM components [51–53]. DPPIV has also been called adenosine deaminase binding protein or adenosine deaminase complexing protein [54]. Chemokines are potential substrates for DPPIV [55], including RANTES (regulated on activation, normal T-cell expressed and secreted) and monocyte chemotactic proteins (MCP) 1, -2, and -3 [56–58] (Table 2). DPPIV expression on T cells has been designated CD26 [42,51]. DPPIV is also expressed on epithelia and melanocytes [42,51,52]. It has long been recognized that expression of DPPIV can be downregulated or altered on cancer cells [59,60]. Specifically, loss or alteration of membrane expression of DPPIV has been reported in prostate [40,61], colorectal [62,63], gastric [62], lung [60], and hepatocellular [60] carcinomas and melanomas [52]. In addition, DPPIV is involved in the fibronectin (FN)-mediated adherence of metastatic breast cancer cells to lung endothelium, in which case DPPIV is expressed on the endothelial cells and FN is expressed on the cell surfaces of the malignant cells [64].

DPPIV expression during malignant transformation has been best characterized in melanocytic cells. A series of work by Houghton and colleagues has shown that DPPIV is expressed in vitro and in vivo by normal melanocytes, but not by melanoma [52,65]. Loss of DPPIV expression probably occurs at an early stage of melanoma progression, when melanocytes transform into melanoma cells [52]. More specifically, DPPIV is expressed by cutaneous melanocytes and common nevi, but is not detected in vivo or in vitro on cells from primary or metastatic melanomas. In an in vitro system that sequentially transformed melanocytes in defined steps, loss of DPPIV expression occurred concomitantly with the emergence of growth factor independence [65,66]. More recently, the re-expression of DPPIV in human melanoma cells at levels comparable with those found in normal melanocytes has been shown to produce profound phenotypic changes [67]. These include abrogation of tumorigenicity, re-emergence of requirements for exogenous growth factors to maintain cell survival, and removal of a block in cell differentiation. Using a point mutation in the active serine protease domain of DPPIV, the authors also observed that serine peptidase activity was required for most effects but not for cell survival. Re-expression of DPPIV rescued expression of a second putative surface peptidase, fibroblast activation protein (FAP/seprase; see below) [68,69], suggesting that expression of this second molecule contributes to effects on cell survival in malignant cells.

7.2. Aminopeptidase N (APN, CD13, EC 3.4.11.2)

The functional role of APN varies depending on its location. Other membrane peptidases, such as NEP or DPPIV often co-localize with APN and seem to cooperate in peptide degradation [42]. Furthermore, like DPPIV [53], APN has been considered an auxiliary adhesion molecule [45].

In contrast to DPPIV, APN is not expressed by normal melanocytes, but becomes increasingly prevalent as melanocytes transform to dysplastic nevocytes and malignant melanoma cells [70,71]. When melanoma cells form colonies, the majority of APN molecules relocate to sites of cell–cell contact; in those cells, APN seems to be tightly associated with ECM components [70]. APN has a direct role in melanoma cell invasion and ECM degradation [50]. Betastatin, a competitive inhibitor of APN function, as well as antibodies to APN, inhibit the penetration of melanoma cells through an artificial basement membrane in vitro without affecting cell adhesion or cell growth [50,70]. Thus, the expression of APN is thought to play a critical role as one member of a cascade of enzymes that hydrolyse extracellular matrix proteins [50,70]. APN may serve as an activator of type IV collagenase and other matrix proteins by cleaving N-terminal amino acids (Fig. 3), thereby allowing the acquisition of invasive and metastatic competence [50,72]. Interestingly, APN was shown to be the major receptor for the transmissible gastroenteritis virus (TGEV) [73] that causes severe gastroenteritis in piglets, and for the human coronavirus 229E [74] that causes upper respiratory infections. More recent data indicate that APN plays an important role in tumor vasculogenesis, identifying it as a critical regulator of angiogenesis [75,76].

7.3. Neutral endopeptidase (NEP, CD10, CALLA, EC 3.4.24.11, enkephalinase, nephrilysin)

Neutral endopeptidase (EC 3.4.24.11), also termed nephrilysin, enkephalinase or CD10 is a 90–110 kDa zinc-dependent metallopeptidase that cleaves peptide bonds on the amino side of hydrophobic amino acids (Fig. 3). It is identical to the common acute lymphoblastic leukemia antigen (CALLA) [77]. NEP inactivates a variety of physiologically active peptides, including neuropeptides, met-enkephalin, substance P, bombesin and endothelin-1, thereby reducing local concentrations of peptides available for receptor binding and signal transduction [78,79] (Table 2). NEP is normally expressed by a wide range of tissues and cells [78].

NEP is also expressed by one-third to one-half of primary and metastatic melanomas and the percentage of NEP-positive cells within a given lesion appears to increase with tumor progression [80]. Thus, unlike other
solid tumor malignancies, melanoma does not fit with the paradigm that NEP is lost upon tumor progression, but that gain of NEP function may be advantageous. More recently, however, it has been reported that NEP is highly expressed by human melanocytes, and that its expression and catalytic activity are downregulated by UVB light. In addition, it has been shown that α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH) are specific substrates for NEP and that specific inhibition of NEP increases the melanogenic activity of these peptides on human melanocytes [81]. Among keratinocyte-derived agents, the melanotropic hormones (α-MSH) and ACTH appear to be very potent stimulators of human pigmentation. These data indicate that NEP inactivation by UVB in melanocytes may enhance the proopiomelanocortin (POMC)-derived peptides paracrine loop, mediating UV-induced pigmentation.

Until now, the biological and regulatory effects of NEP were presumed to result only from its enzymatic function [49]. However, recent data suggest that NEP may possess other biological properties in addition to its ability to catalytically inactivate neuropeptide substrates. NEP protein expression by itself can effect signal transduction pathways, which, in turn, regulate cell growth [82,83] and apoptosis [84].

8. Seprase/fibroblast activating protein: yet another proteolytic enzyme in malignant tumors

A subfamily of membrane-bound nonclassical serine proteases, including seprase and DPPIV, are implicated in matrix degradation and invasiveness of migratory cells [53,85–87]. Seprase is a homodimeric 170-kDa integral membrane gelatinase, whose expression appears to correlate with levels of invasiveness manifested by the human melanoma cell line, LOX, in an in vitro ECM degradation/invasion assay [88]. The deduced amino acid sequence of its 97-kDa subunit (seprase-l), predicts a type II membrane topology with a short cytoplasmic tail (six amino acids) followed by a transmembrane region (20 amino acids) and a large extracellular domain (734 amino acids) [89]. Seprase requires the dimerization of its inactive subunits for activity [89,90]. Comparisons of their deduced amino acid sequences indicate that seprase is essentially identical to human fibroblast activation protein (FAP), which is expressed on reactive stromal fibroblasts of various carcinomas and on fibroblasts of healing wounds [91,92]. Additionally, seprase exhibits a striking sequence homology (52%) to DPPIV, which increases to a 68% amino acid identity between their catalytic regions [89].

Seprase is selectively expressed by fibroblastic cells in areas of active tissue remodeling, such as the embryonic mesenchyme, areas of wound healing, the gravid uterus, and the reactive stroma of epithelial cancers (over 90% of breast, colorectal and lung carcinomas) [91,92]. It is also expressed in vivo in subsets of bone and soft tissue sarcomas [68,69]. This protease is generally absent from the stroma of benign epithelial tumors and normal adult tissues [69]. In vitro, seprase induction is observed in proliferating cultured fibroblasts and in melanocytes grown with basic fibroblast growth factor and phorbol ester [69]. Seprase is a dual-specificity enzyme that acts as a dipeptidyl peptidase and collagenase in vitro [93]. Seprase (−/−) mice are fertile, show no overt developmental defects, and have no general change in cancer susceptibility [94].

9. Ephrins and eph receptors: control of cell behavior by intercellular communication

The Eph receptors are the largest family of receptor tyrosine kinases and include at least 14 structurally related members. Initially isolated as orphan receptors (lacking known ligands), at least eight Eph ligands—ephrins—have recently been reported (Fig. 4) [95,96]. Recent advances have started to elucidate the developmental functions and biochemistry of Eph receptor tyrosine kinases and their membrane-bound ligands, ephrins. Unlike most ligands, ephrins do not function in a soluble form but must be membrane-bound to activate their receptor(s) [95] (Fig. 5).

![Binding specificity classes of ephrins and Eph receptors](image-url)
Fig. 5. Structure, interactions and signal transduction of Eph receptors and ephrins. Eph receptors share a number of features, as indicated. Ephrins have conserved residues in the extracellular domain and fall into two structural classes: proteins of the ephrin-A subclass are anchored in the plasma membrane through the covalent attachment of a glycosylphosphatidylinositol (GPI) group. Proteins of the ephrin-B subclass have a transmembrane domain and short cytoplasmatic region. Bidirectional signaling (arrows) can occur upon interaction of cells expressing Eph receptors and ephrins. Modified from [101,99].

Juxtacrine interactions between Eph (receptor) and ephrin (ligand) on opposing cells were initially implicated in patterning of the brain and somites, and in the process of neural cell guidance (reviewed in [97,98]). Eph receptor tyrosine kinases and ephrins mediate contact-dependent cell interactions that regulate the repulsion and adhesion mechanisms involved in the guidance and assembly of cells, and thus the establishment, maintenance, and remodelling of patterns of cellular organization (reviewed in [95,99]). Eph receptors and ephrins can also trigger an adhesive response of endothelial cells and are required for remodelling of blood vessels (reviewed in [95,100]).

A number of studies have implicated Eph receptors in carcinogenesis based on their elevated expression and/or expression of aberrant transcripts in tumor cell lines [101]. Eph-B3 (Hek-2) and Eph-A2 (Eck) are ectopically expressed in over 90% of melanoma cell lines [102,103]. Cell lines from increasingly advanced melanomas express increasing amounts of Eph-A2 [104]. The first identified Eph ligand was ephrin-A1/B61, a ligand for Ephs including Eph-A2 [105,106]. Ephrin-A1 was found to stimulate proliferation of melanoma cells that overexpress Eph-A2, and therefore, proposed to be an autocrine growth factor [104]. Normal melanocytes do not respond to ephrin-A1 nor express the Eph-A2 receptor. Immunoreactive Eph-A2 was not detectable in most uncultured lesions [107]. However, the ligand ephrin-A1 is expressed by melanoma cells, both in cultured and in uncultured lesions, and correlates with progression [107].

Ephrin-B2 (Lerk-5), a ligand for Eph-B3, is also overexpressed in melanoma and correlates with tumorigenicity and metastatic potential [108]. Eph receptors and ephrins may promote angiogenesis within forming melanoma, or cell–cell repulsion and hence invasion as well as metastatic spread.

10. The ADAM family: multifunctional surface proteins with adhesion and protease activity

The ADAMs are a multifunctional gene family, some of which play a role in diverse biological processes, such as fertilization [109], myogenesis, neurogenesis [110], and the activation of growth factors/immune regulators such as TNF-α [111]. Moreover, the ADAMs have potential implications for tumor metastasis via cell adhesion and protease activities [112,113].

The term ‘ADAM’ stands for a disintegrin and metalloproteinase, which represent the two key structural domains in these molecules. ADAMs, which can process or remove the extracellular domains of cell-surface proteins, are critically placed for regulating signaling. These multifunctional surface proteinases are particularly intriguing in that they contain both cell adhesion and proteolytic domains. The emerging properties of the ADAM gene family have been the subject of several recent reviews [36,37,110,114,115]. Among the 29 known ADAM cDNAs to date, 17 have a metalloproteinase active site.

Cells have the ability to modify their surface to regulate various kinds of functions [37]. For example,
the extracellular domain of > 40 plasma membrane-anchored cytokines, growth factors, receptors, adhesion molecules, and enzymes can be cleaved and thereby released (shed) from the plasma membrane by various proteases (called sheddases or secretases) [116–118]. These sheddases are themselves transmembrane proteins and, in several cases, are ADAMs. One of the best-studied cases of shedding is the release of tumor-necrosis factor α (TNF-α), a cytokine that is involved in the inflammatory response. The release of TNF-α from various cell types occurs in response to injury or infection and plays an important role in the adaptive immune response. TNF-α is synthesized as a 26 kDa membrane-anchored protein from which an active soluble 17 kDa extracellular domain is proteolytically released. This proteolytic release is catalyzed by TNF-α converting enzyme (TACE, or ADAM 17) [116,118].

Kuzbanian (ADAM 19) is also a sheddase that has been found to release a soluble form of delta, Notch ligand [119,120]. Notch is a surface receptor that regulates cell fate determination in various aspects of development, such as neurogenesis [110,117]. Metalloprotease disintegrin cysteine-rich 9 (MDC9, or ADAM 9) has been reported to shed the heparin-binding EGF-like receptor [113]. Both the membrane-anchored and soluble (shed) form of this growth factor are active, but the soluble, diffusible form can act on cells distant from the site of its release. ADAM 17/TACE, ADAM 10/Kuzbanian and ADAM 9/MDC9 are three ADAM metalloproteases for which a function has been reported and that act as sheddases (reviewed in [110]). The other 14 predicted proteases remain ‘orphan proteases’, lacking an identified endogenous substrate [37].

Potentially, cell-surface proteases are also involved in cleavage of growth factors such as TGF-α that are membrane-bound or enzymes and receptors that require activation. At present, no studies have yet comprehensively examined the expression or regulation of ADAMs in melanoma or, for that matter, most solid tumors. However, we anticipate that our understanding of the interplay at the cellular level between melanoma and stromal cells as well as the molecular processes underlying the progression from RGP to VGP melanoma will improve dramatically by continued study of these proteins.

11. Summary and perspective

Our understanding of ECM proteolysis and cell-surface molecules in the progression of melanoma has expanded dramatically in recent years [121]. It is clear that the stroma is an integral part of the tumor and that it contributes to some of the most destructive characteristics of malignant cells [10,38,122]. There is now voluminous evidence that melanoma cells are influenced by the surrounding microenvironment and vice versa [15]. Numerous studies support the concept that melanomagenesis is a multicellular process, in which destruction of the microenvironment is required for the conversion of normal melanocytes to aggressive melanoma cells with the potential for invasion and metastasis.

Understanding the molecular mechanisms by which membrane-bound proteinases are regulated and activated, the nature of their molecular and cellular targets, and how adhesion and proteolysis are integrated will provide exciting new areas for investigation over the coming years and could ultimately lead to novel therapeutical strategies for this aggressive neoplasm. The emerging appreciation of controlled, specific endoproteolytic cleavage of cell-surface receptors to modulate receptor activities and initiate novel signaling pathways also illustrates the complexity of the control mechanisms inherent in the processes of vasculogenesis and angiogenesis [39,95,110]. The molecular mechanisms involved in the complex cross-talk between normal melanocytes as well as melanoma cells and their microenvironment hold great promise as targets for melanoma therapy.

Dissecting the molecular components of melanoma–stroma interactions requires model systems, in which a single variable can be manipulated and assessed. More recently, such powerful models have been emerging, e.g. melanoma in human skin reconstructs or orthotopic in human skin grafted to mice [123]; these will allow us to examine more accurately the pathways and events on the cell-surface and the pericellular space, which impinge on the microenvironment and drive the progression of melanoma to a fatal metastatic neoplasm.

12. Outstanding questions

What features of the microenvironment exactly promote melanoma? Are these melanoma specific?

What is the precise role of cell-surface peptidases and membrane-bound enzymes in regulating the pericellular microenvironment and what are their substrates?

Can changes in the expression of these proteolytic enzymes be used as clinico-pathologic markers for the diagnosis and prognosis of melanoma?

Can the microenvironment be targeted therapeutically to prevent invasive melanoma?

Can manipulating the expression of proteolytic enzymes reverse invasive or metastatic melanoma?
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