ROLE OF AMINopeptidase N (CD13) IN TUMOR-CELL INVASION AND EXTRACELLULAR MATRIX DEGRADATION

Ikuo SAIKI1,2, Hideji FUJI1,2, Junya YONEDA3, Fuminori ABE2, Motowo NAKAJIMA1, Takashi TSURUO1 and Ichiro AZUMA1

1Institute of Immunological Science, Hokkaido University, Kita-15, Nishi-7, Kita-ku, Sapporo 060; 2Research Laboratories, Pharmaceutical Group, Nippon Kayaku Co., Ltd., Kita-ku, Tokyo 115; and 3Institute of Applied Microbiology, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku 113, Tokyo, Japan.

We have investigated the effect of monoclonal antibodies (MAbs) specific for aminopeptidase N/CD13 on the invasion of human metastatic tumor cells into reconstituted basement membrane (Matrigel). The invasion of human metastatic tumor cells (SN12M renal-cell carcinoma, HT1080 fibrosarcoma and A375M melanoma) into Matrigel-coated filters was inhibited by an anti-CD13 MAb, WM15, in a concentration-dependent manner. However, this MAb did not have any effect on tumor-cell adhesion and migration to the extracellular matrices, which may be involved in tumor-cell invasion. MAb WM15 inhibited the degradation of type-IV collagen by tumor cells in a concentration-dependent manner. We also found that WM15 inhibited hydrolysing activities towards substrates of aminopeptidases in 3 different tumor cells. Since our previous study indicated that bestatin, an aminopeptidase inhibitor, was able to inhibit tumor-cell invasion, as well as aminopeptidase activities of murine and human metastatic tumor cells, cell-surface aminopeptidase N/CD13 may be partly involved in the activation mechanism for type-IV collagenolysis to achieve tumor-cell invasion, and anti-CD13 MAb WM15 may inhibit tumor-cell invasion through a mechanism involving its inhibitory action on the aminopeptidase N in tumor cells.

During the metastatic cascade, a tumor cell passes through several connective tissue barriers which consist of various adhesive molecules such as fibronectin, laminin, and other glycoproteins and proteoglycans (Fidler et al., 1978; Liotta et al., 1983; McCarthy et al., 1985; Moscatelli and Rifkin, 1988; Nicolson, 1987; Tryggvason et al., 1987). Tumor invasion is a complex process involving cell adhesion, motility (migration), and the degradation of tissue barriers caused by the different proteases secreted by tumor cells. Therefore, understanding the invasion mechanisms and the control mechanisms of the invasive property of tumor cells may help in the development of anti-metastatic therapies.

Many different types of enzymes that degrade the extracellular matrix in metastatic invasion are not unique to tumor cells and are present in normal tissues and body fluids. However, their expression is controlled under physiological conditions by various growth factors, cytokines and other enzymes. Proteolytic enzymes including lysosomal hydrolases, collagenase, and in some cases a plasminogen activator or plasmin are elevated in invasive cells and tumor cells with metastatic potential (Nakajima and Chop, 1991; Moscatelli and Rifkin, 1988; Nicolson, 1987; Tryggvason et al., 1987). Liotta et al. (1980) have reported that there are substantial correlations between type-IV collagenase (metalloproteinase) activities and metastatic potentials of a variety of human and animal tumor cell lines.

Aminopeptidase N, a surface antigen of many myeloid cells and other diverse cell types, is a well documented ecto-enzyme that binds to the membrane through an N-terminal segment. This enzyme is identical to CD13 (Look et al., 1989) and postulated to perform multiple functions, such as hydrolytic inactivation of regulatory peptides, including enkephalins, that are involved in signal transduction at the cell membrane. Aminopeptidase N/CD13 serves as a receptor for transmissible gastroenteritis virus, and anti-CD13 MAB can block infection by this virus (Delmas et al., 1992; Yeager et al., 1992).

A high level of neutral aminopeptidase activity has been detected on the plasma membrane of several human tumor cell lines and mammalian metastatic tumor cells (Nakajima and Chop, 1991; Chop et al., 1989; Ashmun and Look, 1990; Amòscato et al., 1990). Cell-surface aminopeptidase activity was potently blocked by known inhibitors of the enzyme, such as bestatin and 1,10-phenanthroline (Ashmun and Look, 1990). Bestatin has been shown to possess immunomodulatory effects, presumably through the inhibition of multiple aminopeptidases, including the augmentation of humoral and cell-mediated immune responses and the activation of macrophages and natural killer cells to become cytotoxic against tumor cells (Schlorlemmer et al., 1983; Bruley-Rosset et al., 1979; Saito et al., 1978). Talmadge et al. (1986) have reported that the administration of high doses of bestatin resulted in the significant inhibition of pre-existing experimental and spontaneous metastases in mice. We also demonstrated that bestatin inhibited tumor-cell invasion and the degradation of type-IV collagen by tumor cells, possibly through a mechanism involving its inhibitory action on aminopeptidases in tumor cells (Saiki et al., 1989b; Yoneda et al., 1992).

In the present study, we focused our attention on the role of aminopeptidase N/CD13 on tumor cell surface in the invasion of metastatic tumor cells in vitro.

MATERIAL AND METHODS

Cells

Highly metastatic cell lines, SN12M renal-cell carcinoma (Naito et al., 1989) and A375M melanoma, were kindly provided by Dr. I.J. Fidler, M.D. Anderson Cancer Center, Houston, TX. HT-1080 human fibrosarcoma cells were obtained from the ATCC, Rockville, MD. These cells were maintained as monolayer culture on plastic in Eagle’s minimum essential medium (EMEM) supplemented with 7.5% fetal bovine serum (FBS), vitamin solution, sodium pyruvate, non-essential amino acids and L-glutamine.

Monoclonal antibodies

Monoclonal antibodies (MAbs) against CD13 (human aminopeptidase N) were as follows: WM15 and FITC-labeled WM15 were obtained from Silenus, Howthorn, Australia. My7 (Coulter 6602626) was purchased from Coulter, Hialeah, FL. MCS-2 (MAb specific for human pan-myeloid-monocyte-antigen) was obtained from Nichirei, Tokyo. P1B5 anti-human VLA-3 receptor MAb was purchased from Telios, San Diego, CA. NU-T3 anti-human CD3 MAB was purchased from

4To whom correspondence and reprint requests should be addressed.

Abbreviations: EMEM, Eagle’s minimum essential medium; [125I]IUdR, [125I]iododeoxyuridine; MCA, 4-methoxycoumaryl-7-aminomethylcoumarin; MAB, monoclonal antibody; FITC, fluorescent isothiocyanate.

Received: October 14, 1992 and in revised form December 29, 1992.
Aminopeptidase

Chi methylcoumaryl-7-amides (amino-acid-MCA; Glu-, Ala-, Pro-, Phe-, Gly-, Arg- or Leu-MCA, Peptide Institute, Osaka or Sigma, St. Louis, MO; excitation, 365/380 nm, emission, 450 nm). Reconstituted basement membrane Matrigel (Collaborative Research, Bedford, MA) in a volume of 50 µl. Each assay was performed in triplicate.

Flow-cytometric analysis

Tumor cells were washed with PBS containing 0.2% BSA and 0.1% NaN₃ and suspended in the same buffer. Cell suspensions (10⁶/ml) were added to 96-well microplate wells and incubated for 2 hr at 37°C. Each assay was performed in triplicate.

Immunoelectron microscopy

Sample filters prepared from the invasion assay were embedded in Tissue-Tek (Miles, Elkhart, IN) and blocks were prepared for freezing in liquid nitrogen. Thin frozen sections (15 to 20 nm) were cut on a Histo STAT microtome (Baxter, Mundelein, IL; excitation, 365 nm, emission, 450 nm). The sections were then fixed by immersion for 10 min in 4% paraformaldehyde in buffered saline. Fixed sections were washed 3 times with PBS and 0.1% NaN₃, fixed with methanol and stained with hematoxylin and eosin. The cells on the upper surface of the filters were removed by careful wiping with cotton swabs. The cells that had invaded to various areas of the lower surface were manually counted under a microscope at ×400 magnification. Each assay was performed in triplicate.

Microassay for cell attachment

The cell-attachment assay was carried out as described (Saiki et al., 1989a). Tumor cells in the exponential growth phase were incubated for 24 hr in EMEM containing 5% FBS supplemented with 0.3 µCi/ml [¹²⁵I]iododeoxyuridine ([¹²⁵I]-IUdR) (specific activity: 200 mCi/mmol; NEN, Boston, MA). The filters were washed twice in warm PBS to remove unbound radiolabel, harvested by adding 1 mM EDTA for 1 min at 37°C, and re-suspended in cold serum-free EMEM to form a single suspension of cells. [¹²⁵I]IUdR-labeled tumor cells (2 x 10⁶) were treated with or without MAbs for 30 min, and then the cells were added to microculture wells pre-coated with Matrigel, laminin, fibronectin or bovine serum albumin (BSA). The cultures were incubated at 37°C for 60 min and then washed 4 times with PBS to remove unattached cells. The remaining substrate-bound tumor cells were lysed with 70 µl of 0.1 N NaOH. The lysate was absorbed by cotton swabs and monitored for radioactivity by gamma counting. The binding capacity (number of cells bound/substrate) was expressed as follows:

\[
\text{binding capacity} = \frac{\text{cpm of tumor cells bound to substrate}}{\text{cpm of total tumor cells added}} \times \text{total number of tumor cells added}
\]

Haptotactic migration assay

Tumor-cell migration along a gradient of substrate-bound fibronectin or laminin was assayed in Transwell cell-culture chambers, as reported (Saiki et al., 1989a). The filters were pre-coated with 5 µg fibronectin or laminin in a volume of 50 µl on the lower surface, and dried overnight at room temperature. The coated filters were washed extensively in PBS and then dried immediately before use. The subsequent procedures were the same as those for the invasion assay.

Assay for type-IV collagenolytic activity

In the assay for cell-mediated type-IV collagenolytic activity, tumor cells (5 x 10⁶/well) in Dulbecco’s modified Eagle’s medium (DMEM) and Ham’s nutrient mixture F12 medium (DMEM:F12, 1:1 ratio, Gibco, Grand Island, NY) were added into the wells of a 6-well tissue-culture plate, which had been pre-coated with 1 ml of 1.6 µg/ml N-[propionyl-2-3-¹³C]propionylated collagen (human, type IV, specific activity, 0.014 GBq/mg, NEN). After a 24-hr incubation period, the supernatants were withdrawn and undigested materials were precipitated by mixing them with 100 µl of ice-cold 50% trichloroacetic acid and centrifuged at 18,000 × g for 10 min. Type-IV collagenolytic activity was expressed as net amounts (ng) of degraded type-IV collagen calculated from the radioactivity in the supernatant (Nakajima et al., 1987).

Statistical analysis

The significance of differences between groups was calculated by applying Student’s 2-tailed t-test.

RESULTS

Effect of MAbs specific for aminopeptidase N/CD13 on tumor invasion

We have reported that bestatin, a potent inhibitor of aminopeptidases, including the isolated microsomal and cytosolic leucine aminopeptidase, aminopeptidase B and membrane-bound aminopeptidase, inhibited invasion of murine and human metastatic tumor cells in vitro, as well as the aminopeptidase activity of tumor cells (Saiki et al., 1989b; Yoneda et al., 1992). Therefore, in order to determine the role of a cell-surface aminopeptidase N/CD13 in the tumor inva-
sive process, we examined the effect of 3 different MAbs specific for CD13 on the invasion of human metastatic cells into reconstituted basement membrane Matrigel in vitro. SN12M cells were incubated for 8 hr at 37°C with 3 MAbs specific for CD13 (WM15, My7 and MCS-2) at concentrations ranging from 0.025 to 25 μg/ml in the upper compartment of the Transwell chamber. The results of a representative experiment (out of 3) are shown in Figure 1, and demonstrate that the invasion of SN12M renal carcinoma cells into the Matrigel/

![Image](image.png)

**FIGURE 1** - Effect of anti-CD13 MAbs on the invasion of SN12M cells into reconstituted basement membrane Matrigel. SN12M cells (2 × 10<sup>5</sup>) in 0.1% BSA medium were seeded with or without MAbs specific for CD13 (WM15, My7 or MCS-2) into the upper compartment of a Transwell chamber. Filters in the chamber were pre-coated with 5 μg of Matrigel/fibronectin (■) or Matrigel/laminin (■) on the upper/lower surface, respectively. After an 8-hr incubation, the cells that had invaded through to the lower surface were counted. The assays were performed in triplicate. Bars represent standard deviations. n.t.; not tested; *, p < 0.001.

![Image](image.png)

**FIGURE 2** - Effect of anti-CD13 MAb (WM15) on the invasion of human tumor cells into Matrigel. HT1080 or A375M cells (2 × 10<sup>5</sup>) in 0.1% BSA medium were seeded with or without WM15 onto the Matrigel/fibronectin-coated filters. After an 8-hr incubation, the cells which had invaded through to the lower surface were counted. The assays were performed in triplicate. Bars represent standard deviations. *, p < 0.01; **, p < 0.001.

![Image](image.png)

**FIGURE 3** - Cell-surface expression of aminopeptidase N/CD13 on human metastatic tumor cells. Flow-cytometry analysis was performed on HT1080 (upper panel), SN12M (middle panel) and A375M (lower panel) cells. The cells were treated by a saturating concentration of FITC-conjugated MAb WM15 specific for CD13 (♥) or isotype-matched MAb NU-T3 against human CD3 as a negative control.
cells by flow cytometry after immunofluorescence labeling with the FITC-conjugated MAb WM15 disclosed that 3 tumor cells expressed a moderate or high level of aminopeptidase N at the cell surface (Fig. 3). The distribution of CD13 on the cell surface was also examined by immunoelectron microscopy. Figure 4 shows that CD13 on the SN12M cell surface during the invasion into Matrigel was identified in the invasion edge of tumor cells in the Matrigel and no appreciable staining was observed in the reconstituted basement membrane Matrigel. The above results using one of the anti-CD13 mAbs (WM15) and its specific distribution are associated with the invasion of metastatic tumor cells. However, the level of CD13 expression on the cell surface is distinct from the cell lines used, and is unlikely to correlate with the degree of inhibitory effect on tumor cell invasion by CD13-specific MAb WM15.

Effect of CD13-specific MAb on aminopeptidase activity in tumor cells

We also examined whether or not anti-CD13 MAb (WM15) could inhibit aminopeptidase activity in SN12M cells, by measuring AMC liberated from amino-acid-MCA. As shown in upper panel of Figure 5, SN12M cells showed the various amino-acid-MCA-hydrolysing activities (Ala-, Pro-, Phe-, Gly-, Arg- and Leu-MCA). Ala-MCA was the most sensitive for the hydrolysing activity of tumor cells. The lower panel of Figure 4 shows that MAb WM15 at concentrations ranging from 0.01 to 10 μg/ml inhibited the Ala-MCA-hydrolysing activity of 3 different tumor cells in a concentration-dependent manner, as did bestatin. In contrast, another CD13-specific MAb, MCS-2, which presumably recognizes the different epitopes of aminopeptidase N and showed no inhibition of tumor-cell invasion (Fig. 1), did not inhibit the aminopeptidase activity of tumor cells. The above result is in good agreement with the previous study of Ashmun and Look (1990) on the inhibition of aminopeptidase N by various CD13-specific MAbs (My7 and MCS-2). We have also found that other aminopeptidase inhibitors, amastatin A and arphamenine B, were able to inhibit tumor-cell invasion in vitro (Saiki et al., 1989b). These results demonstrate that MAb WM15 as well as bestatin inhibited the aminopeptidase (metallopeptidase) activity in tumor cells, and that such inhibitory effect was apparently related to the inhibition of tumor-cell invasion into Matrigel.

Effect of CD13-specific MAb WM15 on tumor-cell adhesion and migration

The above findings demonstrated that MAb WM15 inhibited not only the aminopeptidase activity but also the invasion of tumor cells into Matrigel, while other CD13-specific MAbs did not inhibit. Since tumor invasion largely consists of cell adhesion, motility (migration), and the degradation of extracellular matrix and basement membranes by different classes of enzymes, we next examined the effect of MAb WM15 on the adhesion and migration of SN12M tumor cells to the extracellular matrix components. SN12M cells were incubated with 25 μg/ml antibodies for 30 min in the wells pre-coated with extracellular matrix components for the cell-attachment assay. Tumor cells were incubated for 4 hr with antibodies in the upper compartment of the Transwell chamber for the haptotactic migration assay. Table 1 shows that the anti-CD13 MAb WM15 did not inhibit adhesion of SN12M cells to the substrates coated with Matrigel, fibronectin, laminin or BSA.
or haptotactic migration to the filters pre-coated on the lower surface with either fibronectin or laminin. However, MABs specific for VLA-3 integrin receptor as a positive control caused inhibition of tumor-cell migration to the fibronectin-coated filters, as described by Murata et al. (1992). We also observed that the incubation of tumor cells with MABs did not directly affect tumor cell growth and cell viability in vitro (data not shown). The above results indicate that the inhibition of invasion by MAB WM15 might be attributable to the inhibitory effect on the enzymatic degradation of extracellular matrix or basement membranes by tumor cells.

**Effect of anti-CD13 MAB WM15 on enzymatic degradation of extracellular matrices by tumor cells**

Degradation of extracellular matrices during tumor invasion involves a number of enzymes, including collagenase, heparanase and their proteolytic activators. Metalloproteinases capable of specifically degrading type-IV collagen have also been postulated to play a role in tumor-cell invasion and metastasis (Tryggvason et al., 1987; Nakajima et al., 1987; Liotta et al., 1980; Nicolson, 1989). We therefore investigated whether or not a CD13-specific MAB (WM15) is able to influence the degradation of type-IV collagen by tumor cells. SN12M cells were incubated for 24 hr in the presence or absence of MAB WM15 in wells pre-coated with 3H-labeled type-IV collagen. Figure 6 shows that MAB WM15 inhibited the degradation of labeled type-IV collagen by tumor cells in a concentration-dependent manner. In contrast, MAB 84H10 did not inhibit cell-mediated type-IV collagenolysis. We also observed that the conditioned medium from SN12M cells after 24-hr incubation degraded the labeled type-IV collagen, but that MAB 84H10 did not inhibit conditioned-medium-mediated type-IV collagenolysis (data not shown). The results indicate that the CD13-specific MAB WM15 inhibited type-IV collagenolytic activity in the presence of tumor cells, and that the inhibitory effect on tumor invasion by the MAB may be partly attributable to the inhibition of type-IV collagenolysis rather than to the inhibition of tumor-cell attachment and motility in the invasive process. The mechanism for the inhibition of enzymatic degradation by the MAB does not appear to depend on any direct effect against type-IV collagenase.

**DISCUSSION**

We have previously reported that a potent inhibitor of multiple aminopeptidases, bestatin, markedly inhibited the invasion of murine and human metastatic tumors and the degradation of type-IV collagenase by tumor cells but not by tumor-derived conditioned medium (Saiki et al., 1989b; Yonekura et al., 1992). This suggests that the anti-invasive effect by the inhibitor is partly mediated by the inhibitory mechanism for the cell-surface aminopeptidase activity. Aminopeptidase N/CD13 is an important ecto-enzyme and plays physiological roles, not only in myeloid cells but also in cells from diverse tissues (Amoscato et al., 1990; Ashmun and Look, 1990; Kuramochi et al., 1987). Aminopeptidase activity was shown to increase in proportion to the activation and differentiation of macrophages in vitro (Morahan et al., 1980; Wachsmuth, 1975). Gorvel et al. (1990) have reported that neutral aminopeptidases on the surface of granulocytes and macrophages could be associated with the activation of mouse thymic T lymphocytes. However, whether aminopeptidase N/CD13 on normal or malignant cells acts as a regulatory enzyme or serves some other function remains to be determined.

In the present study, in order to extend our previous study on the inhibition of tumor invasion by the aminopeptidase inhibitors, we focused our attention on the role of cell-surface aminopeptidase N/CD13 in the tumor invasive process, and examine the effect of CD13-specific MABs on the invasion of human metastatic tumor cells into the basement membrane. Of 3 MABs specific for CD13, WM15 inhibited the invasion of SN12M, HT1080, or A375M tumor cells into reconstituted basement membrane Matrigel in a concentration-dependent manner, but the other two MABs did not show any invasive property (Figs. 1, 2). The expression of CD13 on the surface of tumor cells was observed by flow cytometry with FITC-labeled WM15 (Fig. 3), and the distribution on the surface was consistently located in the invasive processes (Fig. 4). The level of CD13 expression on the surface of different tumor cells is not necessarily associated with the degree of the inhibition of tumor-cell invasion by WM15. Aminopeptidase activity in tumor cells was inhibited to a similar degree by the addition of WM15 as well as of bestatin, but was not inhibited by another anti-CD13 MAB, MCS-2 (Fig. 5). The above results are well consistent with the previous reports on the inhibition of aminopeptidase N by various anti-CD13 MABs (Ashmun and Look, 1990).
Look, 1990). Thus, the inhibitory effect on tumor invasion is likely to be in parallel with inhibition of the aminopeptidase activity by the MAb. The penetration of tumor cells into basement membranes involves distinct events, including attachment of tumor cells, secretion by the tumor cells of enzymes that cause degradation of the adjacent basement membrane, and migration of the cells into the target tissue. However, the CD13-specific MAb WM15 did not affect tumor-cell attachment to extracellular matrix components or the motility (haptotactic migration) of tumor cells to fibronectin or laminin substrates (Table 1), nor did it affect the growth of tumor cells (data not shown). Therefore, the anti-invasive effect of WM15 may be associated with the degradative cascade of extracellular matrices.

Metalloproteinase activity of degrading basement membrane collagen type IV was first reported by Liotta et al. (1979). Good correlation between type-IV collagenase activity and the metastatic potential of tumor cells has been found using a variety of human and animal tumor lines (Nakajima et al., 1987; Liotta et al., 1980). Anti-CD13 MAb (WM15) inhibited the degradation of type-IV collagen by SN12M cells in a concentration-dependent manner (Fig. 6). This result is in good agreement with our previous findings that bestatin, an aminopeptidase inhibitor, inhibited type-IV collagenolysis by tumor cells (Saiki et al., 1986b; Yoneda et al., 1992). Chop et al. (1989) found high levels of expression of plasma-membrane-associated aminopeptidase activity in rat mammary adenocarcinoma cells, which spontaneously metastasize from rat mammary-fat-pad sites to the lymph nodes and the lung. Thus, aminopeptidase activity on metastatic tumor cells may be relevant to the cascade of tumor-invasion mechanisms by hydrolysing extracellular matrix components already cleaved by other proteinases, such as plasmin or type-IV collagenase (Nakajima and Chop, 1991). However, our previous study using the zymography of SN12M-derived conditioned medium showed that the treatment of tumor cells with bestatin resulted in the disappearance of the 68-kDa type-IV collagenase level (active form) and slight reduction of the 72-kDa type-IV collagenase level (latent form) (Yoneda et al., 1992). We also found that bestatin did not inhibit the hydrolyzing activity of tumor-derived plasmin (Yoneda et al., 1992), which can degrade basement-membrane components and activate the latent collagenase produced by tumor cells (Liotta et al., 1981). These results using anti-CD13 MAb WM15 as well as bestatin indicate that plasma-membrane aminopeptidase N/CD13 may be involved in the activation or conversion mechanisms of type-IV collagenase and other matrix proteinases such as stromelysin, possibly by removing N-terminal amino acid and initiating or completing activation processes. The role of membrane-bound aminopeptidases, including CD13, in tumor invasion and metastasis remains to be determined in detail.

In conclusion, we demonstrated that aminopeptidase N/CD13-specific MAb potently inhibited tumor-cell invasion and the degradation of type-IV collagen by tumor cells through the mechanism based on the inhibition of tumor-derived aminopeptidase-N activity.

ACKNOWLEDGEMENTS

This work was supported in part by grants-in-aid for cancer research from the Japanese Ministry of Education, Science and Culture; from the Japanese Ministry of Education, Science and Culture; from the Japanese Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control; and for Scientific Research from the Japanese Ministry of Education, Science and Culture; and also by the Osaka Foundation for the Promotion of Clinical Immunology. The authors thank Dr. Y. Nodasaka for technical assistance in electron microscopy, and Ms. M. Sato for typing the manuscript.

REFERENCES

Amoscato, A.A., Stankowski, R.M., Babcock, G.F. and Alexander, J.W., Neutral surface aminopeptidase activity of human tumor cell lines. Biochem. biophys. Acta, 1041, 317–319 (1990).

Ashmun, R.A. and Look, A.T., Metalloproteinase activity of CD13/aminopeptidase N on the surface of human myeloid cells. Blood, 75, 462–469 (1990).

Bruley-Rosset, M., Florentin, I., Kinger, N., Schutz, J. and Mathe, G., Restoration of impaired immune functions of aged animals by chronic bestatin treatment. Immunology, 38, 75–83 (1979).

Chop, A.M., Nakajima, M. and Nicolson, G.L., Metastatic mammary adenocarcinoma cell lines expressed elevated levels of plasma membrane cathespins-H-like and aminopeptidase-like activities. Proc. Am. Assoc. Cancer Res., 30, 98 (1989).

Delmas, B., Gueff, J., L'Haridon, R., Vogel, K.L., Siostrom, H., Noren, O. and Laude, H., Aminopeptidase N is a major receptor for the entropathogenic coronavirus TGEV. Nature (Lond.), 357, 417–420 (1992).

Fidler, I.J., Gersten, D.M. and Hart, J.R., The biology of cancer invasion and metastasis. Advanc. Cancer Res., 28, 149–259 (1978).

Gorvel, J.-F., Vivier, I., Naquet, P., Brekelmans, P., Regal, A. and Perret, M., Characterization of the neutral aminopeptidase activity associated to the mouse thymocyte-activating molecules. J. Immunol., 144, 2899–2907 (1990).

Kuramochi, H., Motegi, A., Iwashishi, M., Takahashi, K., Horinshi, H. and Umezawa, H., Action of ubiquinone on aminopeptidase activities in spleen cells and peritoneal macrophages from mice. J. Antibiotics, 40, 1605–1611 (1987).

Liotta, L.A., Abe, S., Gehron, P.R. and Martin, G.R., Preferential digestion of collagen matrix by an enzyme derived from metastatic tumor. Proc. natl. Acad. Sci. (Wash.), 76, 2268–2272 (1979).

Liotta, L.A., Goldfarb, R.H., Brundage, R., Siegal, G.K., Terranova, V., Qureshi, S., Effect of aminopeptidase inhibitors on the aminopeptidase activity of tissue plasminogen activator and plasmin, and in inhibition of glycoprotein and collagenous components of basement membrane. Cancer Res., 41, 4629–4636 (1981).

Liotta, L.A., Rao, C.V. and Barsky, S.H., Tumor invasion and the extracellular matrix. Lab. Invest., 49, 639–649 (1983).

Liotta, L.A., Tryggsason, K., Garbina, S., Hart, I., Foutz, C.M. and Shafie, S., Metastatic potential correlates with enzymatic degradation of basement-membrane collagen. Nature (Lond.), 284, 67–68 (1980).

Look, T.A., Ashmun, R.A., Shapiro, L.H. and Peiper, S.C., Human melanoma plasma membrane aminopeptidase CD13 (gp150) is identical to aminopeptidase N. J. clin. Invest., 83, 1299–1307 (1989).

McCarthy, J.B., Basara, M.L., Palm, S.L., Sats, D.F. and Furcht, L.T., The role of cell-adhesion proteins—laminin and fibronectin—in the movement of malignant and metastatic cells. Cancer Metast. Rev., 4, 125–132 (1985).

Morahan, P.S., Edelson, P.J. and Grinn, K., Changes in macrophages epoxygenes associated with anti-tumor activity. J. Immunol., 125, 1312–1317 (1980).

Moscatelli, D. and Rifkin, D.B., Membrane and matrix localization of proteinases—a common theme in tumor-cell invasion and angiogenesis. Biochem. biophys. Acta, 948, 67–85 (1988).

Murata, J., Saki, I., Yoneda, J. and Azuma, I., Differences in chemotaxis to fibronectin in low- and high-metastatic tumor cells. Jap. J. Cancer Res., 83, 1327–1333 (1992).

Naito, S., Walker, S.M. and Fidler, I.J., In vivo selection of human renal-cell carcinoma cells with high metastatic potential in nude mice. Clin. exp. Metast., 7, 381–389 (1989).

Nakajima, M. and Chop, A.M., Tumor invasion and extracellular matrix degradative enzymes: regulation of activity by organ factors. Semin. Cancer Biol., 2, 115–127 (1991).

Nakajima, M., Welch, D.R., Bulloni, P.N. and Nicolson, G.L., Degradation of basement-membrane type IV collagen and lung subendothelial matrix by rat mammary adenocarcinoma cell clone of differing metastatic potential. Cancer Res., 47, 4869–4876 (1987).

Nicolson, G.L., Tumor-cell instability, diversification, and progres-
INVOLVEMENT OF AMINOPEPTIDASE IN TUMOR INVASION

Sion to the metastatic phenotype' from oncogene to oncofetal expression. Cancer Res., 47, 1473–1487 (1987).

NICOLSON, G.L., Metastatic tumor-cell interactions with endothelium, basement membrane and tissue. Curr. Opinion Cell Biology, 1, 1009–1019 (1989).

SAIKI, I., IIDA, J., MURATA, J., OGAWA, R., NISHI, N., SUGIMURA, K., TOKURA, S. and AZUMA, I., Inhibition of the metastasis of murine malignant melanoma by synthetic polymeric peptides containing core sequence of cell-adhesive molecules. Cancer Res., 49, 3815–3822 (1989a).

SAIKI, I., MURATA, J., WATANABE, K., FUJII, H., ABE, F. and AZUMA, I., Inhibition of tumor-cell invasion by ubenimex (bestatin) in vitro. Jap. J. Cancer Res., 80, 873–878 (1989b).

SAITO, K., TAKEGOSHI, K., AOYAGI, T., UMEZAWA, H. and NAGAI, Y., Stimulatory effect of bestatin, a new specific inhibitor of aminopeptidase, on the blastogenesis of guinea-pig lymphocytes. Cell Immunol., 40, 242–262 (1978).

SCHORLEMMER, H.-U., BOSSLLET, K. and SEDLACEK, H.H., The ability of the immunomodulating dipeptide bestatin to activate cytotoxic mononuclear phagocytes. Cancer Res., 43, 4148–4153 (1983).

TALMADGE, J.E., LENZ, B.F., PENNINGTON, R., LONG, C., PHILIPS, H., SCHNEIDER, M. and TRIBBLE, H., Immunomodulatory and therapeutic properties of bestatin in mice. Cancer Res., 46, 4505–4510 (1986).

TRYGGVASON, K., HOYHTYA, M. and SALO, T., Proteolytic degradation of extracellular matrix in tumor invasion. Biochem. biophys. Acta, 907, 191–217 (1987).

WACHSMUTH, E.D., Aminopeptidase as a marker for macrophage differentiation. Exp. Cell Res., 96, 409–412 (1975).

YEAGER, C.L., ASHMUN, R.A., WILLIAM, R.K., CARDELLICHO, C.B., SHAPIRO, L.H., LOOK, A.T. and HOLMES, K.Y., Human aminopeptidase N is a receptor for human coronavirus 229E. Nature (Lond.), 357, 420–422 (1992).

YONEDA, J., SAIKI, I., FUJII, H., ABE, F., KOJIMA, Y. and AZUMA, I., Inhibition of tumor invasion and extracellular matrix degradation by ubenimex (bestatin). Clin. exp. Metastasis, 10, 49–59 (1992).