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REVIEW

Role of Matrix Metalloproteinases and Their Inhibitors in Tumor Invasion and Metastasis

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Abstract—The role of various matrix metalloproteinases (MMP)—such as gelatinases, stromelysins, matrilysin, collagenase-3, and membrane-bound MMP (MB-MMP)—in tumor invasion and metastasis is discussed. Data suggesting significance for malignant growth of the expression level of these enzymes and also of their activators and inhibitors are presented. It is concluded that at different stages of tumor progression the activity of different MMPs is displayed, which is regulated by various growth factors and oncogenes. Different malignancies are characterized by changes in activities of specific MMPs. Data are presented which show significance of the ratio between the MMP activity and that of tissue inhibitors of metalloproteinases (TIMP) in tumor invasion and metastasis, especially in connection with a dual role of TIMP as both MMP inhibitors and activators.

Key words: matrix metalloproteinases, membrane-bound metalloproteinases, tissue inhibitors of metalloproteinases, invasion and metastasis of tumor cells

A regulated degradation of the intercellular matrix is necessary for the normal course of various physiological processes such as embryogenesis, morphogenesis, angiogenesis, tissue involution, and wound healing [1, 2]. The progress of many diseases including arthritis, glomerulonephritis, atherosclerosis, peptic ulcer, periodontal diseases, pneumosclerosis, autoimmune diseases, and also invasion of tumor cells and metastasis [2-11] is associated with disorders in the regulation of the intercellular matrix degradation. In addition to other proteinases, an important role in this process is played by enzymes of the matrix metalloproteinase (MMP) family, which can destroy proteins of the intercellular matrix and basal membranes: collagens, proteoglycans, elastin, laminin, fibronectin, etc. [12-14]. At present, about 20 enzymes of this family are described, as well as four representatives of the family of MMP inhibitors, tissue inhibitors of MMP (TIMP).

Because destruction of basal membranes or the adjacent connective tissue is required for invasion and migration of tumor cells and the subsequent metastasis, changes in the expression levels of MMP, their activators, and inhibitors are significant for development of malignancies [15, 16].

The cells of tumor and adjacent tissues synthesize or induce synthesis of a number of enzymes including MMP which seem to remove the physical barrier for migration of tumor cells due to proteolysis of macromolecules of the extracellular matrix, such as collagens, laminins, fibronectins, and proteoglycans, and also by influencing cell adhesion [16]. In turn, products of matrix degradation influence differentiation and proliferation and, thus, are involved in early stages of tumor progression [10, 16, 17].

Consider the involvement in tumor invasion and metastasis of the best studied members of the MMP family: gelatinases, stromelysins, matrilysin, collagenase-3, and membrane-bound MMP (MB-MMP).

Gelatinases. Many experimental data suggest that gelatinases capable of destroying type IV collagen are involved in degradation of basal membranes, which occurs in the course of invasion on the boundary between the tumor and normal tissues [10]. Thus, a high expression level of mRNA for MMP-2 and MMP-9 and also activities of these proteinases were found during the progress of mammary gland carcinoma [18-20]. MMP-2 was already shown to be one of the key enzymes in invasion and metastasis of various tumors. Immunochemical studies of MMP-2 location in cells of rectal carcinoma revealed this enzyme on the invasive boundaries of the carcinoma, and the corresponding mRNA was found in the stroma cells. These findings suggest that the activation of MMP-2 on the tumor cell surface should immediately influence the ability of the carcinoma cells for invasion [21]. MMP-2 was shown to bind to vitronectin receptors on the surface of invasive cells [22], and such location determines its ability to influence cell growth and differ-

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entiation [23]. In the case of human head and neck squamous carcinoma, the level of proMMP-2 activation in the tumor tissue significantly determines the appearance of metastases in lymph nodes [24].

There are many reports indicating that disorders in the regulation of secretion and activity of gelatinase B also can play a role in invasion of tumor cells and metastasis [25]. A direct correlation is found between increase in the expression level of MMP-9 in the blood vessel tissue of patients with blood cancer and the severity of disease [26]. Moreover, the *in vivo* growth of glioblastoma was shown to be accompanied by increase in MMP-9 production [27].

The expression of MMP-2 was found immunohistochemically in both tumor and stroma cells of human neuroblastomas, whereas the expression of MMP-9 was found in the stroma cells and in vascular and perivascular cells surrounding groups of the tumor cells [28]. High activities of these enzymes corresponded to a high degree of the tumor metastasis.

Stromelysins. Stromelysin-1 is especially interesting: its expression is usually absent in steady state tissues, but it is easily induced by cytokines, growth factors, and tumor promotors [13, 29]. Its activity is increased in tissues of stomach carcinoma [30]. A high activity of another member of this MMP subfamily, stromelysin 3, is observed in invasive mammary gland carcinomas [18, 31, 32]. Its increased expression is also found in cells of other human carcinomas: head and neck squamous carcinoma [32, 33] and skin and rectal tumors [32, 34]. And the content of mRNA for stromelysin-3 is the highest in cells of tumors with high local invasiveness. Some authors believe the expression of stromelysin-3 in stroma fibroblasts to be controlled by factors produced by the tumor cells [35].

Matrilysin. An important role in tumor invasion is also played by matrilysin (MMP-7) due to its ability to destroy type IV collagen, laminin, and entactin of basal membranes [36-40]. The expression of mRNA for matrilysin is increased in carcinomas of prostate [41], stomach, rectum [42], head and neck, and lungs [43] compared to normal tissues surrounding these tumors. The activity of matrilysin was found in more than 75% of tissue specimens of human tumors of mammary gland, large intestine, stomach, prostate, esophagus, and upper respiratory tracts [44].

The ability of matrilysin to destroy the fibrillar form of fibronectin can be especially significant for migration of tumor cells. Degradation of fibronectin under the influence of matrilysin results in production of specific fragments which seem to be involved in the regulation of gene expression, ligand-receptor recognition, and other processes. By blocking specific receptors of intact fibronectin, proteolytic fragments of fibronectin can weaken the attachment of tumor cells to the connective tissue stroma and thus promote their migration [45].

Transfection of human hepatic cell carcinoma (HCC) cells with the gene *Ets-1* of the Ets family of transcription factors increased the expression of matrilysin, whereas the transfection with a corresponding antisense oligonucleotide decreased it. These findings suggest that at least in HCC cells the expression of MMP-7 is under the control of this oncogene [46].

Collagenase-3. The activity of collagenase-3 (MMP-13) was first detected in mammary gland carcinoma. Later, many tumors were found to produce this enzyme [2, 47, 48]. Collagenase-3 can destroy fibrillar collagens and display gelatinolytic activity when involved in the degradation of collagen fragments as a result of collagenolysis [49]. The substrate specificity of collagenase-3 is also extended to proteoglycans, aggrecan [50], and many other proteins of the intercellular matrix, including tenascin C, fibronectin, fibrillin, and the type IV, IX, X, and XIV collagens [51, 52]. The expression of collagenase-3 plays a role in the development of many malignant tumors in humans: carcinomas of head and neck, skin, and mammary gland, and chondrosarcoma [2]. The activity of collagenase-3 was found in 30% of mammary gland carcinomas, whereas such activity was absent in benign tumors and normal tissue of mammary gland [47]. By *in situ* hybridization, transcripts of mRNA for collagenase-3 were found mainly in stroma cells surrounding epithelial cells of the tumor [53]. Moreover, fibroblast cells with an increased expression of mRNA for collagenase-3 were adjacent to the cells located on the invasive boundary of the tumor. Although in some cases of mammary gland carcinoma the expression of collagenase-3 was found in epithelial tumor cells [54], stroma cells are thought to play the main role in production of this enzyme [2].

Role of membrane-bound MMP in tumor invasion and metastasis. Notwithstanding active studies on these metalloproteinases, their functions and substrate specificity remain unclear. MB-MMPs play a dual role in the regulation of degradation and remodeling of the matrix. On one hand, they are proteolytic enzymes, and gelatin, fibronectin, B-chain of laminin, vitronectin, dermatan sulfate proteoglycan, and collagens are their substrates [55, 56]. On the other hand, they activate progelatinase A [57, 58] and collagenase-3 [49] on the cell surface. The expression of MB-MMPs seems tissue-specific: the presence of MB1-MMP and MB2-MMP is specific for many adult human tissues, whereas the distribution of MB3-MMP, MB4-MMP, and MB5-MMP is more limited, in particular, their expression is found in brain cells [59].

MB1-MMP is located on the surface of tumor cells and seems to influence their invasion. An increased expression of this enzyme is observed in various tumor tissues [30, 60, 61]. Studies of Nomura et al. [30] have shown a direct correlation between the MB1-MMP expression in the cells of stomach tumor and the activation of MMP-2 which accompanies invasion and metas-

tasizing of tumors. New data favoring the involvement of MB1-MMP in tumor invasion were obtained by Kadono et al. [62]. They showed that transformation of the Madin Darby line of dog kidney epithelial cells (MDCK) with the oncogene $p60^{v\text{-}src}$ changes the cell morphology and results in the loss of intercellular contacts and increased tumorigenicity. Concurrently the MB1-MMP gene expression is induced, and the active form of progelatinase A appears in the culture medium.

Expression of other MMPs (MB2-MMP [61] and MB4-MMP [63]) is observed in the cells of mammary gland carcinoma.

An increased production of proMMP-2 and the rate of its activation in some malignant tumors of human brain (astrocytoma, glioblastoma) directly correlate with expression of MB1- and MB2-MMP [64], which also suggests their roles in the activation of proMMP-2 and tumor invasion. Progelatinase A can also be activated during the growth of human brain tumors by recently found metalloproteinases, such as MB5-MMP [65] (the content of its transcript is increased in various astrocytomas and glioblastomas), and MB6-MMP [59], which is also expressed in some tumors of human brain and in SW480 cells of intestine carcinoma. It should be emphasized that expression of MB6-MMP has not been found in normal cells of brain and intestine [59]. It seems that the malignant transformation is associated with the expression of MB-MMP nonspecific for normal tissues, but progelatinase A in various tumors is activated by different MB-MMP.

Cooperation of tumor cells with the adjacent stroma cells promotes the invasiveness and facilitates metastasis, and this is clearly exemplified by expression of MB1-MMP. According to [66], in the case of mammary gland and lung tumors mRNA for MB1-MMP is expressed in fibroblasts which are in contact with the malignant cells. Different levels of MB1-MMP expression in fibroblasts and tumor cells seem to be specific for different stages of development of the invasive phenotype. In the first stage of tumor invasion, the expression of MB1-MMP by stroma fibroblasts is induced by various factors secreted by the adjacent tumor cells. On acquisition of a complete metastatic phenotype, they began to produce MB1-MMP. Some stroma cells in the pre-invasive and invasive tumor zones are also shown to express mRNA for MB1-MMP, progelatinase A, and TIMP-2. The increased expression of MB1-MMP and TIMP-2 activates gelatinase A and provides for a subsequent degradation of the matrix.

Martorana et al. studied cooperation of different cells using the cell line (BC1) of metastasizing carcinoma of rat mammary gland which includes two subpopulations of cells, epithelial and metaplastic ones, with characters of mesenchymal cells [67]. The metaplastic cells were shown to express mRNA for MMP under the influence of factors secreted by the epithelial cells. Such cooperation seems important for the progress of neoplastic tumors that are phenotypically heterogeneous [67].

Regulation of MMP expression. The mechanisms of regulation of MMP expression in norm and during tumor progression remain unclear. However, some cytokines, growth factors, and oncogenes (transcription factors) have been found which influence the synthesis and activation of MMP. Some data suggest that a high level of MMP-2 expression is induced by the interaction of two transcription factors, AP2 and YB-1 [68]. Expression of the *H-ras* gene induces increased activity of MMP-2 and the invasiveness of the MCF10A line of epithelial cells of human mammary gland which is assessed by invasion into the matrigel [69]. The insulin-like growth factor 1 (IGF-1) also seems to play a certain role in the regulation of MMP-2 expression. Lung carcinoma cells of Lewis strain mice with a high metastasizing potential and with IGF-1 receptor expression prevented by antisense-mRNA displayed a significantly decreased invasiveness into the reconstructed basal membrane than the control. By polymerase chain reaction these cells were shown to have a sixfold lower content of mRNA transcripts for MMP-2 and a correspondingly decreased amount of MMP-2 that was determined by Western-blotting and zymography. Respectively, the increased expression of the IGF-1 receptor in another cell line with low ability for metastasis significantly increased the content of MMP-2 and of the corresponding mRNA [70].

By *in situ* hybridization, the expression of various MMPs was shown to depend on the type of cells producing the tumor of human mammary gland and on the stage of the tumor development [54]. The authors suggested that the expression of most members of the MMP family is regulated by signals entering from the host's cells in response to the tumor invasion. TGF-β, which differently influences the expression of different MMPs, is especially interesting. The expression of collagenase-3 in some malignant cells is under positive control of such factors as TGF-β, IL-1, and bFGF. The induction of this enzyme under the influence of TGF-β or TGF-α in transformed keratinocytes has been recently found to be mediated by a protein kinase activated by a mitogen p38 [2]. The invasiveness of the Detroit-562 cell line of squamous carcinoma increases under the influence of EGFR, which increases the rate of synthesis and activation of MMP-9 [71].

A high level of expression of collagenase-3 in the cells of various human carcinomas can be associated with the presence in the gene of this enzyme of the neighboring sequences AP-1 and PEA-3, the combination of which provides for a special sensitivity of the expression to growth factors, products of oncogenes, and tumor promotors [2]. The majority of genes for MMP have a similar structure of these sites: AP-1 and PEA-3 in the promotor. A specific interaction between these *cis*-elements and different combinations of nuclear oncoproteins

encoded by Fos, Jun, and Ets can be significant for the positive and negative regulation of MMP synthesis on the transcription level in normal and neoplastic tissues. An increased expression of MMP in tumor cells seems to be a result of inactivation of inhibitory factors specific for the differentiated phenotype and activation of the activating factors specific for non-differentiated cells. In particular, Jun B of the Jun family of transcriptional factors, which suppress the transcription of MMPs, is suggested to be such a factor [72]. The authors suggest that c-Ets-1 of the Ets family of transcription factors activates the transcription of MMP. It is likely that dedifferentiation of epithelial tumors is due to the loss by the cells of the Jun B activity or to the increase in the activity of c-Ets-1, or to a combination of these two processes. As mentioned above, the increased expression of the *Ets-1* oncogene in the hepatic carcinoma cells was associated with an increased expression of matrilysin [46]. Transfection of the cells with an appropriate antisense oligonucleotide results in suppression of the Ets-1 expression and decrease in the transcription of mRNA for matrilysin that suggests an involvement of Ets-1 in the regulation of synthesis of matrilysin. However, the expression of matrilysin in different tumors can be also controlled by other factors. β-Catenin, a component of the caderin complex which is involved in intercellular contacts, was shown to activate the expression of matrilysin in stomach tumor cells [73].

Proteins encoded by oncogenes of the *ras* family regulate the expression of many genes [74]. It is now established that transcription of the gene encoding MMP-9 is under positive control of *ras* family genes [75-77]. A negative regulation of the MMP-9 expression can be realized with the involvement of the *RECK* gene, which encodes a membrane-bound glycoprotein [78]. This is shown by data indicating that the recovery of the gene *RECK* expression in the oncogene-transformed cells suppresses their ability for invasion along with a decrease in the secretion of MMP-9.

Role of TIMP in tumor invasion and metastasis. Disorders in the balance between the activities of MMP and TIMP resulting in an excess degradation of the matrix are specific for tumor invasion and metastasis [75, 79]. However, data on the expression of TIMP in various tumor cells are rather contradictory and, therefore, can not be interpreted unambiguously. Thus, studies on the expression of mRNA for TIMP-1 and TIMP-2 in human tumors have shown increased levels of these mRNAs. In the cells of mammary gland carcinoma the expression level of mRNA for TIMP-1 was significantly higher than in adjacent normal tissues [80]. The content of mRNA for TIMP was also high in the tissues surrounding blood vessels of the tumor and in the stroma of mammary gland tumor, especially in the region of active fibrogenesis on the stroma boundary [81]. In tissues of malignant ovary tumors the increased expression of TIMP-1 correlated with a high activity of MMP-9 typical for these tumors [82].

A high level of the TIMP-1 expression is specific for progressing stages in development of some lymphomas [83, 84]. A correlation is observed between an increase in the TIMP-2 expression and high rate of relapses of mammary gland carcinoma [85] and also between the expression of mRNA for TIMP-2 and the progress of colorectal carcinoma and blood cancer [86, 87]. By *in situ* hybridization, a prevalent expression of the TIMP-3 gene in the stroma of mammary gland carcinoma is shown at the boundary surface with the tumor cells [88].

Development of gene therapy approaches seems promising for application of MMP inhibitors for suppression of tumor invasion and metastasis. By experiments with the transfer of TIMP genes into cells, the importance of TIMP for invasion and metastasis was confirmed. Thus, transfection of the TIMP-1 gene into tumor cells, such as B16-F10 melanoma transformed by the rat embryonal *c-H-ras* oncogene, human stomach cancer, and astrocytoma, decreased the ability of these cells for invasion and metastasis [89-92]. Similar results were obtained by transfection of transformed cells with the TIMP-2 gene [89]. It was shown on the model of colorectal cancer in hairless mice that the transfection with the adenovirus vector of the TIMP-2 gene into hepatocytes and the subsequent expression of TIMP-2 prevented malignant transformation and decreased the growth rate of already existent metastasizing foci in the liver [93]. However, although the increased expression of TIMP-2 by a cell line of human melanoma suppressed the local growth of the tumor, the spontaneous metastasis was not decreased [94]. Moreover, the cells of mouse epidermal tumor transfected with the TIMP-3 gene displayed increased tumorigenicity and invasiveness [95]. It was suggested that TIMP-3 should promote the detachment of the transformed cells from the extracellular matrix, which was shown to accelerate morphological changes associated with the transformation [96].

On the other hand, the increased expression of TIMP-1 and TIMP-2 suppressed the invasion of melanoma cells and the *in vivo* and *in vitro* tumor growth [90, 94, 97]. According to [98], the increased expression of TIMP-3 also suppressed the *in vivo* tumor growth and induced the death of tumor cells in suspension. In experiments described in [99] the increased expression of TIMP-3 suppressed more effectively than TIMP-1 and TIMP-2 the invasion of melanoma cells across reconstructed basal membranes and also caused apoptosis. Other authors [100] suggest that the loss by the cells of capacity to synthesize TIMP-3 can increase the invasiveness of some tumors. The transfection of the TIMP-3 gene into the cells of leukomyosarcoma SK-LMS-1 line resulted in changes in the cell morphology, growth rate, and binding with the matrix, and these changes could be considered as suppression of the invasive phenotype under the influence of TIMP-3.

Transfection of the human TIMP-1 gene in the cells of rat mammary gland carcinoma increased the production of laminin and the type IV collagen, not affecting the activity of MMP [101]. Thus, a high content of endogenous TIMP-1 can stimulate the *in vivo* growth of tumor by stimulating the production of the basal membrane matrix components.

Interaction of tumor and host cells is especially important for tumor progress, invasion, and metastasis. Many MMP are expressed in the cells of the tumor stroma, and this confirms the significant role of the latter in the tumor growth and progress [17]. Thus, MMP-2 was shown to be secreted as a proenzyme by the stroma fibroblasts surrounding the cells of human head and neck carcinoma and activated under the influence of MB1-MMP located in the tumor cells [24]. Induction of the synthesis and activation of MMP in the tumor stroma and adjacent normal tissues is likely to be a direct or indirect response to the presence of the tumor cells and occurs under the influence of signal molecules synthesized by the host's cells. The metastatic phenotype of the tumor is produced depending on the close interaction between the tumor and stroma cells, and the activity of different MMP is specifically manifested in different stages of this process. The increased expression of TIMP is a response to the tumor progress directed to suppress the activity of MMP and provide for the integrity of the extracellular matrix. However, based on the dual role of the inhibitor TIMP-2, which together with MB1-MMP activates progelatinase A, it is suggested that the inhibitor/activator ratio should determine the tumor growth and metastasis.

In conclusion, it should be said that further studies in this line should promote the development of approaches for antitumor therapy based on inhibitors of MMP.

REFERENCES

1. Birkedal-Hansen, H., Moore, W. G. I., Bodden, M. K., Windsor, L. J., Birkedal-Hansen, B., deCarlo, A., and Engler, J. A. (1993) *Crit. Rev. Oral. Biol. Med.*, **42**, 197-250.
2. Pendas, A. M., Uria, J. A., Jimenez, M. G., Balbin, M., Freije, J. P., and Lopez-Otin, C. (2000) *Clin. Chim. Acta*, **291**, 137-155.
3. Murphy, G., and Hembry, R. M. (1992) *J. Rheumatol.*, **19**, 61-64.
4. Galis, Z. S., Sukhova, G. K., Kranzhofer, R., Clark, S., and Libby, P. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 402-406.
5. Nicolson, G. L. (1991) *Semin. Cancer. Biol.*, **2**, 143-154.
6. Docherty, A. J. P., O'Konnell, J., Crabbe, T., Angal, S., and Murphy, G. (1992) *Trends Biotechnol.*, **10**, 200-207.
7. Mignatti, P., and Rifkin, D. B. (1993) *Physiol. Rev.*, **73**, 161-195.
8. Opdenakker, G., and van Damm, J. (1994) *Immunol. Today*, **15**, 103-107.
9. Woessner, J. F., Jr. (1994) *Ann. N. Y. Acad. Sci.*, **732**, 11-21.
10. MacDougall, J., and Matrisian, L. M. (1995) *Cancer Metastasis Rev.*, **14**, 351-362.
11. Nagase, H. (1997) *Biol. Chem.*, **378**, 151-160.
12. Woessner, J. F. (1991) *FASEB J.*, **5**, 2145-2154.
13. Matrisian, L. M. (1992) *BioEssays*, **14**, 455-463.
14. Kleiner, D. E., Jr., and Stetler-Stevenson, W. G. (1993) *Curr. Opin. Cell Biol.*, **5**, 891-897.
15. Kähäri, V.-M., and Saarialho-Kere, U. (1999) *Ann. Med.*, **31**, 34-45.
16. Kleiner, D. E., and Stetler-Stevenson, W. G. (1999) *Cancer Chemother. Pharmacol.*, **43** (Suppl.), S42-S51.
17. Roskelley, C. D., Srebrow, A., and Bissell, M. J. (1995) *Curr. Opin. Cell Biol.*, **7**, 736-747.
18. Wolf, C., Rouyer, N., Lutz, Y., Adida, C., Loriot, M., Bellocq, J.-P., Chambon, P., and Basset, P. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 1843-1847.
19. Davies, B., Miles, D. W., Happerfield, L. C., Naylor, M. S., Bobrow, L. G., Rubens, R. D., and Balkwill, F. R. (1993) *Br. J. Cancer*, **67**, 1126-1131.
20. Soini, Y., Hurskainen, T., Hoyhtyä, M., Oikarinen, A., and Autio-Harmainen, H. (1994) *J. Histochem. Cytochem.*, **42**, 945-951.
21. Poulsom, R., Pignatelli, M., Stetler-Stevenson, W. G., Liotta, L. A., Wright, P. A., Jeffery, R. E., Longcroft, J. M., Rogers, L., and Stamp, G. W. (1992) *Am. J. Pathol.*, **141**, 389-396.
22. Brooks, P. C., Stromblad, S., Sanders, L. C., von Schalscha, W. G., Aimes, R. T., Stetler-Stevenson, W. G., Quigley, J. P., and Cheresh, D. A. (1996) *Cell*, **85**, 683-693.
23. Turck, J., Pollock, A. S., Lee, L. K., Marti, H.-P., and Lovett, D. H. (1996) *J. Biol. Chem.*, **271**, 15074-15083.
24. Tokumaru, Y., Fujii, M., Otani, Y., Kaneyama, K., Imanishi, Y., Igarashi, N., and Kanzaki, J. (2000) *Cancer Lett.*, **150**, 15-21.
25. Himelstein, B. P., Canete-Soler, R., Benhardt, E. J., Dilks, D. W., and Muschel, R. J. (1995) *Invasion Metastasis*, **14**, 246-258.
26. Bianco, F. J., Jr., Gervasi, D. C., Tiguert, R., Grignon, D. J., Pontes, J. E., Crissman, J. D., Fridman, R., and Wood, D. P., Jr. (1998) *Clin. Cancer Res.*, **4**, 3011-3016.
27. Sawaya, R., Go, Y., Kyritisis, A. P., Uhm, J., Venkaiah, B., Mohanam, S., Gokaslan, Z. L., and Rao, J. S. (1998) *Biochem. Biophys. Res. Commun.*, **251**, 632-636.
28. Siguira, Y., Shimada, H., Seeger, R. C., Laug, W. E., and deClerc, Y. A. (1998) *Cancer Res.*, **58**, 2209-2216.
29. McDonell, S., Wright, J. H., Gaire, M., and Matrisian, L. M. (1994) *Biochem. Soc. Trans.*, **22**, 58-63.
30. Nomura, H., Sato, H., Seiki, M., Mai, M., and Okada, Y. (1995) *Cancer Res.*, **55**, 3263-3266.
31. Basset, P., Bellocq, J. P., Wolf, C., Stoll, I., Hutin, P., Limacher, J. M., Podhajcer, O. L., Chenard, M. P., Rio, M. C., and Chambon, P. (1990) *Nature*, **348**, 699-704.
32. Okada, A., Bellocq, J., Chenard, M. P., Rio, M. C., Chambon, P., and Basset, P. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 2730-2734.
33. Muller, D., Wolf, C., Abecassis, J., Millon, R., Engelmann, A., Bronner, G., Rouyer, N., Rio, M.-C., Eber, M., Methlin, G., Chambon, P., and Basset, P. (1993) *Cancer Res.*, **53**, 165-169.
34. Majmudar, G., Nelson, B. R., Jensen, T. C., Voorhees, J. J., and Johnson, T. M. (1994) *Mol. Carcinogenesis*, **9**, 17-23.

35. Noël, A. C., Lefebvre, O., Maquoi, F., VanHoorde, L., Chenard, M.-P., Mareel, M., Foidart, J.-M., Basset, P., and Rio, M.-C. (1996) *J. Clin. Invest.*, **97**, 1924-1930.
36. Quantin, B., Murphy, G., and Breathnach, R. (1989) *Biochemistry*, **28**, 5327-5334.
37. Miyazaki, K., Hattori, Y., Umenishi, R., Yasumitsu, H., and Umeda, M. (1990) *Cancer Res.*, **50**, 7758-7764.
38. Sires, U. I., Griffin, G. L., Broekelmann, T. J., Mecham, R. P., Murphy, G., Chung, A. E., Welgus, H. G., and Senior, R. M. (1993) *J. Biol. Chem.*, **268**, 2069-2074.
39. Murphy, G., Cockett, M. I., Ward, R. V., and Docherty, A. J. P. (1991) *Biochem. J.*, **277**, 277-279.
40. Mayer, U., Mann, K., Timpl, R., and Murphy, G. (1993) *Eur. J. Biochem.*, **217**, 877-884.
41. Pajouh, M. S., Nagle, R. B., Breathnach, R., Finch, J. S., Brawer, M. K., and Bowden, G. T. (1991) *J. Cancer Res. Clin. Oncol.*, **117**, 144-150.
42. McDonnell, S., Navre, M., Coffey, R. J., and Matrisian, L. M. (1991) *Mol. Carcinogenesis*, **4**, 527-553.
43. Muller, D., Breathnach, R., Engelmann, A., Millon, R., Bronner, G., Flesch, H., Dumont, P., Eber, M., and Abecassis, J. (1991) *Int. J. Cancer*, **48**, 550-556.
44. Wilson, C. L., and Matrisian, L. M. (1996) *Int. J. Biochem. Cell Biol.*, **28**, 123-136.
45. Von Bredow, D. C., Nagle, R. B., Bowden, G. T., and Cress, A. E. (1995) *Exp. Cell Res.*, **221**, 83-91.
46. Ozaki, I., Mizuta, T., Zhao, G., Yotsumoto, H., Hara, T., Kajihara, S., Hisatomi, A., Sakai, T., and Yamamoto, K. (2000) *Cancer Res.*, **60**, 6519-6525.
47. Freije, J. M. P., Diez-Itza, I., Balbin, M., Sanchez, L. M., Blasco, R., Tolivia, J., and Lopez-Otin, C. (1994) *J. Biol. Chem.*, **269**, 16766-16773.
48. Lopez-Otin, C. (1999) *Collagenase-3: Identification, Characterization and Physiological and Pathological Relevance. Collagenases* (Hoeffler, W., ed.) Landes Company, Austin, Texas, pp. 37-53.
49. Knäuper, V., Lopez-Otin, C., Smith, B., Knight, G., and Murphy, G. J. (1996) *J. Biol. Chem.*, **271**, 1544-1550.
50. Fosang, A. J., Last, K., Knäuper, V., Murphy, G., and Neame, P. J. (1996) *FEBS Lett.*, **380**, 17-20.
51. Knäuper, V., Cowell, S., Smith, B., Lopez-Otin, C., O'Shea, M., Morris, H., Zardi, L., and Murphy, G. J. (1997) *J. Biol. Chem.*, **272**, 7608-7616.
52. Answorth, J. L., Murphy, G., Rock, M. J., Sherratt, J., and Shapiro, S. D. (1999) *Biochem. J.*, **340**, 171-181.
53. Uria, J. A., Stahle-Backdahl, M., Seiki, M., Fueyo, A., and Lopez-Otin, C. (1997) *Cancer Res.*, **57**, 4882-4888.
54. Heppner, K. J., Matrisian, L. M., Jensen, R. A., and Rodgers, W. H. (1996) *Am. J. Pathol.*, **149**, 273-282.
55. Pei, D., and Weiss, S. J. (1996) *J. Biol. Chem.*, **271**, 9135-9140.
56. Ohuchi, E., Imai, K., Fujii, Y., Sato, H., Seiki, M., and Okada, Y. (1997) *J. Biol. Chem.*, **272**, 2446-2451.
57. Sato, H., Takino, T., Kinoshita, T., Imai, K., Okada, Y., Stetler-Stevensen, W. G., and Seiki, M. (1996) *FEBS Lett.*, **385**, 238-240.
58. Kinoshita, T., Sato, H., Takino, T., Itoh, M., Akizawa, T., and Seiki, M. (1996) *Cancer Res.*, **56**, 2535-2538.
59. Velasco, G., Cal, S., Merlos-Suáres, A., Ferrando, A. A., Alvarez, S., Nakano, A., Arribas, J., and López-Otín, C. (2000) *Cancer Res.*, **60**, 877-882.
60. Sato, H., Takino, T., Okada, Y., Cao, J., Schinagawa, A., Yamamoto, E., and Seiki, M. (1994) *Nature*, **370**, 61-65.
61. Ueno, H., Nakamura, H., Inoue, M., Imai, K., Noguchi, M., Sato, H., Seiki, M., and Okada, Y. (1997) *Cancer Res.*, **57**, 2055-2060.
62. Kadono, Y., Okada, Y., Namiki, M., Seiki, M., and Sato, H. (1998) *Cancer Res.*, **58**, 2240-2244.
63. Puente, X. S., Pendas, A. M., Llano, E., Velasco, G., and Lopez-Otin, C. (1996) *Cancer Res.*, **56**, 944-949.
64. Nakada, M., Nakamura, H., Ikeda, E., Fujimoto, N., Yamashita, J., Sato, H., Seiki, M., and Okada, Y. (1999) *Am. J. Pathol.*, **154**, 417-428.
65. Llano, E., Pendás, A. M., Freije, J. P., Nakano, A., Knäuper, V., Murphy, G., and López-Otin, C. (1999) *Cancer Res.*, **59**, 2570-2576.
66. Polette, M., and Birembaut, P. (1998) *Int. J. Biochem. Cell Biol.*, **30**, 1195-1202.
67. Martorana, A. M., Zheng, G., Crowe, T. C., O'Grady, R. L., and Lyons, J. G. (1998) *Cancer Res.*, **58**, 4970-4979.
68. Mertens, P. R., Alfonso-Jaume, M. A., Steinmann, K., and Lovett, D. H. (1998) *J. Biol. Chem.*, **273**, 32957-32965.
69. Moon, A., Kim, M. S., Kim, T. G., Kim, S. H., Kim, H. E., Chen, Y. Q., and Kim, H. R. (2000) *Int. J. Cancer*, **85**, 176-181.
70. Long, L., Navab, R., and Brodt, P. (1998) *Cancer Res.*, **58**, 3243-3247.
71. O-Charoenrat, P., Modjtahedi, H., Rhys-Evans, P., Court, W. J., Box, G. M., and Eccles, S. A. (2000) *Cancer Res.*, **60**, 1121-1128.
72. Crawford, H. C., and Matrisian, L. M. (1996) *Enzyme Protein*, **49**, 20-37.
73. Crawford, H. C., Fingleton, B., Rudolf-Owen, L. A., Goss, K. J., Rubinfeld, B., Polakis, P., and Matrisian, L. M. (1999) *Oncogene*, **18**, 2883-2891.
74. Hunter, T. (1997) *Cell*, **88**, 333-346.
75. Liotta, L. A., Steeg, P. A., and Stetler-Stevenson, W. G. (1991) *Cell*, **64**, 327-336.
76. Stetler-Stevenson, W. G., Hewitt, R., and Concoran, M. (1996) *Semin. Cancer Biol.*, **7**, 147-154.
77. Himelstein, B. P., Lee, E., Sato, H., Seiki, M., and Muschel, R. J. (1997) *Oncogene*, **14**, 1995-1998.
78. Takahashi, C., Sheng, Z., Horan, T. P., Kitayama, H., Maki, M., Hitomi, K., Kitaura, Y., Takai, S., Sasahara, R. M., Horimoto, A., Ikawa, Y., Ratzkin, B. J., Arakawa, T., and Noda, M. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 13221-13226.
79. Tanaka, K., Iwamoto, Y., Ito, Y., Ishibashi, T., Nakabeppu, Y., Sekiguchi, M., and Sugioka, Y. (1995) *Cancer Res.*, **55**, 2927-2935.
80. Yoshiji, H., Gomez, D. E., and Thorgeirsson, U. P. (1996) *Int. J. Cancer*, **69**, 131-134.
81. Lindsay, C. K., Thorgeirsson, U. P., Tsuda, H., and Hirohashi, S. (1996) *Hum. Pathol.*, **28**, 359-365.
82. Kikkava, F., Tamakoshi, K., Nawa, A., Shibata, K., Yamagata, S., Yamagata, T., and Suganuma, N. (1997) *Cancer Lett.*, **120**, 109-115.
83. Kossakowska, A. E., Urbanski, S. J., and Edwards, D. R. (1991) *Blood*, **77**, 12475-12481.
84. Kossakowska, A. E., Urbanski, S. J., Watson, A., Hayden, L. J., and Edwards, D. R. (1993) *Oncol. Res.*, **5**, 19-28.

85. Visscher, D. W., Höyhtyä, M., Ottosen, S. K., Liang, C. M., Sarkar, F. H., Crissman, J. D., and Fridman, R. (1994) *Int. J. Cancer*, **59**, 339-344.
86. Grignon, D. J., Sakr, W., Toth, M., Ravery, V., Angulo, J., Shamsa, F., Pontes, J. E., Crissman, J. C., and Fridman, R. (1996) *Cancer Res.*, **56**, 1654-1659.
87. Urbanski, S. J., Edwards, D. R., Hershfield, N., Huchcroff, S. A., Shaffer, E., Sutherland, L., and Kossakowska, A. E. (1993) *Diagn. Mol. Pathol.*, **2**, 81-89.
88. Byrne, J. A., Tomasetto, C., Rouyer, N., Bellocq, J. P., Rio, M. C., and Basset, P. (1995) *Mol. Med.*, **1**, 418-427.
89. DeClerck, Y. A., Perez, N., Shimada, H., Boone, T. C., Langley, K. E., and Taylor, S. M. (1992) *Cancer Res.*, **52**, 701-708.
90. Khokha, R., Zimmer, M. J., Graham, C. H., Lala, P. K., and Waterhouse, P. (1992) *J. Natl. Cancer Inst.*, **84**, 1017-1022.
91. Tsuchiya, Y., Sato, H., Endo, Y., Okada, Y., Mai, M., Sasaki, T., and Seiki, M. (1993) *Cancer Res.*, **53**, 1397-1402.
92. Matsuzawa, K., Fukuyama, K., Hubbard, S. L., Dirks, P. B., and Rutka, J. T. (1996) *J. Neuropathol. Exp. Neurol.*, **55**, 88-96.
93. Brand, K., Baker, A. H., Perez-Canto, A., Possling, A., Sacharjat, M., Geheeb, M., and Arnold, W. (2000) *Cancer Res.*, **60**, 5723-5730.
94. Montgomery, A. M. P., Mueller, B. M., Reisfeld, R. A., Taylor, S. M., and deClerck, Y. A. (1994) *Cancer Res.*, **54**, 5467-5473.
95. Sun, Y., Kim, H., Parker, M., and Stetler-Stevenson, W. G. (1996) *Anticancer Res.*, **16**, 1-7.
96. Yang, T. T., and Hawkes, S. P. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 10676-10680.
97. Imren, S., Kohn, D. B., Shimada, H., Blavier, L., and deClerck, Y. A. (1996) *Cancer Res.*, **56**, 2891-2895.
98. Brian, J., Wang, Y., Smith, M. R., Kim, H., Jacobs, C., Jakman, J., Kung, H. F., Colburn, N. H., and Sun, Y. (1996) *Carcinogenesis*, **9**, 1805-1811.
99. Ahonen, M., Baker, A. H., and Kähäri, V. M. (1998) *Cancer Res.*, **58**, 2310-2315.
100. Castagnino, P., Soriano, J. V., Montesano, R., and Bottaro, D. P. (1998) *Oncogene*, **17**, 481-492.
101. Yoshiji, H., Buck, T. B., Harris, S. R., Ritter, L. M., Lindsay, C. K., and Thorgeirsson, U. P. (1998) *Biochem. Biophys. Res. Commun.*, **247**, 605-609.