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TUMOR CELL INTERACTIONS WITH THE EXTRACELLULAR MATRIX DURING INVASION AND METASTASIS

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INTRODUCTION

Malignancy is defined as neoplastic growth that tends to metastasize. Thus by definition metastatic ability is the correlate of malignant potential. The formation of metastatic foci is the most life-threatening aspect of malignant neoplasia. Occult metastatic tumor cells may persist in a dormant state for years after the resection or elimination of the primary tumor (Meltzer 1990; Zajicek 1987). They can then be activated by as yet unidentified stimuli and metastatic foci suddenly develop in an explosive fashion, which results in a rapid demise of the cancer patient. Most cancer deaths are due to the metastatic disease that remains resistant to conventional therapies. The primary aim of research into the mechanisms of tumor invasion and metastasis formation is to identify new strategies for more effective therapy against this most deadly aspect of human cancer.

The study of the genetic alterations associated with human tumor progression has yielded great insight into the mechanisms of oncogenesis (Bishop 1991; Fearon & Vogelstein 1990; Fidler & Radinsky 1990). Studies have clearly shown that the tumor development (tumorigenicity) and subsequent metastatic behavior (malignant potential) are under separate genetic control (Garbisa et al 1987; Muschel et al 1985). Genetic studies have demonstrated that establishment of a primary tumor focus is the result of multiple genetic alterations leading to uncontrolled tumor cell growth. These alterations include the loss or inactivation of anti-oncogenes as well as the activation of cellular proto-oncogenes. The genetic alterations associated with oncogenic transformation may occur in a random order and the resulting tumorigenicity is the net sum of these genetic changes. To date a single gene has not been identified that regulates the entire metastatic process. However, this is not surprising if we consider the multistep nature of metastasis formation, and that the genetic approach requires isolation and independent characterization of the multiple genetic alterations that may occur at each step.

SELECTION OF THE METASTATIC SUBPOPULATION: Clonal Dominance

Successful metastasis formation involves a series of linked sequential steps. This process requires a single tumor cell or groups of tumor cells to dissociate from the primary tumor, invade the surrounding extracellular matrix, including both basement membranes and interstitial compartments, enter the vascular or lymphatic space, escape immune surveillance and mechanical disruption, arrest at a distant site, escape from the vascular or lymphatic circulation, penetrate the target tissue and proliferate as a secondary colony

that may itself subsequently metastasize. The fully competent metastatic tumor cell is capable of successfully negotiating all the steps in this sequence (Fidler & Radinsky 1990). Failure may occur at any step, which results in complete loss of metastatic behavior and elimination of the tumor cells. In fact, only a very small percentage of tumor cells that reach the circulation will survive and form metastases. Further complicating the study of metastatic phenotype development is the assumption that competency for each step of the cascade may be developed independently, in a random and reversible fashion. Although it is probable that genetic alterations may be associated with each individual step, the failure to clearly identify metastasis genes common in all tumors with this phenotype suggests that many of the alterations associated with metastatic cells may be quantitative in nature.

Fidler & Hart (1982) originally described tumor metastasis as a highly selective competition favoring the survival of a subpopulation of metastatic cells preexistent within the heterogeneous population of the primary tumor. This original view remains valid, and the metastatic subpopulation idea of Fidler is well-established by the consistent manner in which subclones of varying metastatic potential can be isolated from primary cultures of human tumors. Kerbel and colleagues (Kerbel 1990), using genetic markers for clonality, demonstrated that the metastatic subpopulation dominates the primary tumor mass early in its growth. More recently these investigators have shown that factors that behave as growth inhibitors for early stage benign tumors can switch function and act as mitogens for tumor cells of more advanced stages of disease progression, e. g. when tumor cells attain metastatic competence (Cornil et al 1991; Kerbel 1992). This effect confers a selective growth advantage to the small numbers of metastatically competent cells present within the primary tumor and accounts for the observed clonal dominance of these metastatic cells in the growing primary and at distant metastatic foci.

TUMOR-HOST INTERACTIONS MODULATE THE METASTATIC PROCESS

Orthotopic implantation of human colon carcinoma cells into nude mice demonstrates that the organ environment clearly influences the metastatic capacity of the injected tumor cells (Fidler 1991; Fidler et al 1990; Nakajima et al 1990; Stephenson et al 1992). Regardless of the malignant potential of the tumor cells in the original human patient, the transplanted human colon tumor cells did not metastasize unless they were injected into the cecum or spleen of the nude mouse. Heterotopic subcutaneous implantation resulted in a much lower frequency of metastasis formation. Recent experiments demonstrate that orthotopic implantation of human tumor cells from

breast, stomach, pancreas, and prostate into nude mice favors recapitulation of the metastatic behavior and pattern of metastasis seen for these tumors in their human hosts (Fidler 1991; Fidler et al 1990; Furukawa et al 1993; Manzotti et al 1993; Nakajima et al 1990, Stephenson et al 1992). Further enhancement of this behavior was seen when fragments of tumor tissue were placed at the orthotopic site rather than a suspension of human tumor cells passaged in vitro (Furukawa et al 1993; Stephenson et al 1992). These findings suggest that in addition to tumor cell-host interactions the organization of tumor cells within the tumor itself and interaction with tumor-derived matrix may also influence metastatic behavior.

DEFINITION OF THE INVASIVE/METASTATIC PHENOTYPE: The Expanded Three-Step Hypothesis

Many of the steps in metastasis formation require specific interactions with the extracellular matrix, and the nature and degree of these matrix interactions will change from step to step during the metastatic process. Only the metastatically competent cells will successfully navigate each of these interactions. Escape of tumor cells from the primary tumor may require decreased adhesiveness to the tumor or stromal matrix. However, the arrest in the target organ that results in tumor-specific patterns of metastasis formation may be mediated by specific tumor-endothelial interactions and selective binding to specific matrix components (Pauli et al 1990; Zhu et al 1991). Tumor cells also may respond differently to various extracellular matrices and stromal cells that are encountered during metastasis formation. The concept of dynamic reciprocity, i.e. normal cells that produce extracellular matrix are also influenced by that matrix (Sage & Bornstein 1991), is also valid for tumor cells and the extracellular matrices that they encounter. However, the responses of tumor cells to various matrix components may also be anomalous when compared with that of normal cells, just as malignant tumor cell response to growth factors may be paradoxical when compared with that of benign or normal cells (Kerbel 1992).

Malignant tumor cell interactions with the extracellular matrix are different from those of normal or benign cells. The behavior of the malignant tumor cell is characterized by its tendency to cross tissue boundaries, intermix with cells of the various compartments, and metastasize to distant sites. It is now recognized that this invasive behavior is also shared by a number of normal cell types and occurs to a limited degree in other physiologic and pathologic conditions (Mareel et al 1990). For example, trophoblasts invade the endometrial stroma and blood vessels to establish contact with the maternal circulation during development of the hemocorial placenta.

Endothelial cells must invade basement membranes and interstitial stroma during angiogenesis. The striking similarity at the molecular level between these processes leads us to suggest that the ability of tumor cells to cross multiple tissue boundaries is the result of loss of control over the expression of the invasive phenotype observed in these normal cells (Liotta et al 1991). The invasive phenotype of tumor cells may be viewed as a quantitative escalation, or loss, of regulation over the normal invasive behavior of activated endothelial cells or trophoblasts, and acquisition of this phenotype is essential for successful completion of many steps in the metastatic cascade.

A successful strategy for studying the invasive phenotype has been to define and examine individual steps within this process. Historically, tumor cell interaction with the basement membrane is defined as the critical event of tumor invasion that signals the initiation of the metastatic cascade (Fidler & Radinsky 1990; Liotta et al 1991). The apparent emphasis on interaction of tumor cells with the basement membrane arises because of the presence of these connective tissue barriers at multiple key points in the metastatic cascade: escape from the primary tumor in epithelial malignancies, intravasation and extravasation during hematogenous dissemination, and perineural and muscular invasion. These basement membranes, composed of a dense meshwork of collagen-type IV, laminin, and heparan sulfate proteoglycans, do not normally contain pores that would allow passive tumor cell migration. Tumor cell traversal of basement membrane barriers is the result of acquisition of an invasive phenotype that can be separated into three steps: attachment, local proteolysis, and migration. However, it should be emphasized that these three steps describe tumor cell interaction with all extracellular matrices and not just basement membranes. The nature of the specific interaction (i.e. tumor cell types and type of matrix) may result in emphasis of some steps over others at particular points in the metastatic cascade. It is through the repetitive cycling of these three steps that tumor cells accomplish many of the processes necessary for successful metastatic behavior.

Metastasis is therefore a multistep process involving numerous tumor cell-host interactions. These interactions are defined by the invasive phenotype that is dominated by the ability of tumor cells to attach to the extracellular matrix, to degrade matrix components, and then migrate through these matrix defects. None of these functions is unique to tumor cell behavior. The difference between normal invasive processes and the pathologic nature of tumor cell invasion is therefore one of regulation. An understanding of the factors that control cellular processes essential to the invasive phenotype should allow identification of new therapeutic targets for prevention and treatment of metastasis formation.

CELL ADHESION DURING INVASION AND METASTASIS

A number of specific cell-surface-associated molecules that modulate cell-matrix and cell-cell interactions have been characterized. These include the integrins, a 67 kd laminin-binding protein, cadherins, Ig superfamily, and CD44. The role of these classes of molecules in tumor growth, invasion, and metastasis is under active investigation. As mentioned previously, tumor cells must show both decreased cell and matrix adhesive properties as well as enhancement of these functions at various stages of the metastatic process. Therefore, the apparent contribution of each class of cell adhesion molecules to the net cellular and matrix adhesiveness of tumor cells will be dependent on a variety of factors including the metastatic capacity of the tumor cell population under study and the model system used to study these cells.

Integrins are a family of cell-surface receptors that mediate cell adhesion. At least twenty different integrins have been characterized to date. They are formed by various noncovalent associations of 14 α and 8 β subunits to form heterodimers (Albelda 1993; Hynes 1992; Ruoslahti & Pierschbacher 1987). The integrins were originally identified as receptors for extracellular matrix proteins such as collagens, fibronectin, laminin, and vitronectin. Some integrins may also function as cell-cell adhesion molecules. Work has shown considerable redundancies within the integrin family in that most of the integrins bind to more than one ligand, and ligands can be recognized by more than one integrin.

The effects of integrin function on invasion and metastasis have been examined. A number of integrins bind via recognition of the RGD sequence common to a number of adhesive molecules including fibronectin, vitronectin, and other adhesion proteins (Albelda 1993; Ruoslahti & Pierschbacher 1987; Yamada 1991). The receptor functions associated with these integrins can be inhibited by synthetic RGD-containing peptides. These peptides have been used to disrupt integrin functions and successfully inhibit both in vitro and in vivo melanoma cell invasion (Gehlsen et al 1988; Humphries et al 1986, 1988; Saiki et al 1989). However, relatively high peptide concentrations (from 100–1000 $\mu g\ ml^{-1}$ for in vitro assays; 0.2–3 mg per injection with in vivo models) are required to achieve these effects. Recent work has shown a similar inhibitory activity of RGD-containing peptides, the so-called dysintegrins, obtained from snake venom.

Other evidence shows that expression of the vitronectin receptor ($a_v\beta_3$ integrin) is elevated in malignant melanoma cells (Albelda et al 1990; Gehlsen et al 1992). This receptor is also overexpressed in glioblastoma multiforme (Gladson & Cherech 1991). Most recently, experiments with the human melanoma cell line A375M have revealed that the enhanced

ability of these cells to invade basement membranes in response to treatment with anti-$a_v\beta_3$ antibodies may be related in part to an increased expression of the matrix-degrading enzyme, 72-kd type IV collagenase (gelatinase A) (Seftor et al 1992). Signal transduction through this vitronectin receptor may modulate proteolytic enzyme production to enhance the invasive phenotype.

Cell surface receptors for laminin may mediate adhesion of tumor cells to the basement membrane prior to invasion (Rao et al 1982; Wewer et al 1986). Laminin is known to play a key role in cell attachment, cell spreading, mitogenesis, neurite outgrowth, morphogenesis, and cell movement. Many types of neoplastic cells contain cell-surface-binding sites for laminin with affinity constants in the nanomolar range. The isolated laminin receptor is a 67-kd protein that binds to the B chain (short arm) region of the laminin molecule (Wewer et al 1986, 1987). Breast carcinoma and colon carcinoma tissue contain a higher number of unoccupied receptors compared with benign lesions. The 67-kd laminin receptors of normal epithelium are polarized at the basal surface and occupied with laminin in the basement membrane. In contrast, the 67-kd laminin receptors on invading carcinoma cells are amplified and dispersed over the entire surface of the cell. Laminin adhesion can be shown experimentally to play a role in hematogenous metastasis (Barsky et al 1984; Liotta 1986). Pretreatment of tumor cells with very low concentrations of the receptor-binding fragment from the laminin molecule markedly inhibited or abolished the formation of lung metastasis from tumor cells injected intravenously. Recent studies using the MLuC5 monoclonal antibody, which is specific for the 67-kd laminin receptor, have shown that expression of this marker is associated with poor prognosis in human breast cancer patients (Martignone et al 1993). Multivariant analysis showed that the 67-kd laminin receptor is an independent prognostic factor, which indicates its predictive value in relation to overall survival.

Recent studies have indicated an inhibitory role for cell adhesion molecules (CAMs) of the cadherin family in the process of metastasis. Cadherins are calcium ion-dependent CAMs that mediate cell-cell binding (Takeichi 1990). Three subtypes (E-, N-, and P-cadherins) have been identified in mammals, and they are primarily distinguished by tissue distribution. E-cadherin, also termed uvomorulin, plays an anti-metastatic role in vitro with epithelial Madin-Darby canine kidney (MDCK) cells (Behrens et al 1989; Uleminckx et al 1991). When treated with monoclonal anti-E-cadherin antibodies, these cells acquired in vitro invasive capability (Takeichi 1990). Further studies manipulating the expression of E-cadherin have been informative (Uleminckx et al 1991). Transfection of plasmids containing the sense strand of E-cadherin messenger RNA in highly invasive clones resulted in overexpression

of E-cadherin protein and the loss of invasive capacity. The partial down-regulation of E-cadherin by transfection of anti-sense mRNA in a noninvasive clone resulted in the acquisition of invasive behavior. These results indicate that enhancing the ability of tumor cells to bind to one another or to other host cells inhibits the ability of tumor cells to escape from their primary site to initiate invasion. Therefore, these studies provide evidence that E-cadherin can act as an invasion suppressor molecule.

Other cell adhesion molecules include the Ig superfamily members such as N-CAM, and VCAM-1. The Ig superfamily incorporates a wide variety of proteins that all share the immunoglobulin homology unit, which consists of 70 to 110 amino acids organized into 7–9 β-sheet structures (Albelda 1993). Family members include molecules involved in a variety of cell functions including cellular immunity and signal transduction, as well as cell adhesion (Hunkapiller & Hood 1989; Williams & Barclay 1988). This diversity makes generalizations about the role of Ig superfamily members in tumor cell invasion difficult. However, the role of VCAM-1 in facilitating the metastatic process appears straightforward. VCAM-1 (also known as INCM-110) was identified on endothelial cells as a cytokine-inducible, counter-receptor for the VLA-4 ($\alpha_4\beta_1$) integrin (Elices et al 1990; Osborn et al 1989; Rice & Bevilacqua 1989). VLA-4 is found primarily on white blood cells and functions in mediating leukocyte-endothelial cell attachment. Recently it was shown that malignant melanoma cells may also express VLA-4 (Albelda et al 1990; Osborn et al 1989). Thus VCAM-1 may serve as a tumor adhesion receptor, facilitating interaction of circulating melanoma cells with the endothelium in advance of tumor cell extravasation.

Enhancement of tumor cell attachment may also be facilitated by CD44. CD44 is a widely distributed integral membrane protein that appears to function as a receptor for hyaluronic acid (Aruffo et al 1990; Culty et al 1990; Gallatin et al 1991). It exists in a variety of M_r forms ranging from 85 to 160 K. A 90 K form found on leukocytes is thought to be important in lymphocyte homing. Larger isoforms are found on epithelial and mesenchymal cells. The epithelial form was more highly expressed in carcinoma, and overexpression was also seen in widely disseminated large-cell lymphomas (Horst et al 1990; Stamenkovic et al 1989). In another study, melanoma cells were sorted by fluorescence-activated cell sorting (FACS) analysis using CD44 antibodies (Birch et al 1991). Melanoma cells expressing higher CD44 levels resulted in significantly more lung colonies following intravenous injection into the nude mouse. These studies suggest that enhanced tumor cell binding to hyaluronic acid may effect tumor cell implantation and is important for attainment of full metastatic competency.

Altered tumor cell-matrix interactions may also contribute to the phenotypic changes associated with invasion. Kleinman and colleagues have shown

that nontumorigenic NIH-3T3 cells will become tumorigenic, locally invasive, and form highly vascularized tumors following co-injection of the cells with reconstituted basement membrane, matrigel (Fridman et al 1992b). Investigators have also shown that a number of human tumor cell lines that are nontumorigenic in the nude mouse will become tumorigenic if co-injected with matrigel (Fridman et al 1991). The specific components of the basement membrane responsible for these effects have not been directly identified. However, a 19 residue synthetic peptide derived from the laminin A chain appears to enhance the metastatic phenotype of murine melanoma cells in vivo (Kanemoto et al 1991). The active sequence within this peptide has been identified as the hexapeptide sequence SIKVAV, which induces proteolytic activity and angiogenesis normally associated with the invasive phenotype (Sweeney et al 1991).

Recent work has shown that tumor cells and normal cells may be differentiated in vitro by the nature of their interactions with reconstituted basement membranes. Reconstituted basement membrane (matrigel) was used to culture normal and malignant breast cells and tissues (Petersen et al 1992). The normal cells were distinguished by their ability to re-express a differentiated phenotype as evidenced by formation of true acini within the matrix. Human breast cancer cell lines were easily distinguished by their lack of polarity and lack of a continuous basement membrane. As suggested, this system could be used to define and identify tumor supressor genes involved in preserving normal cell-matrix interaction and communication.

Thus the current evidence regarding the involvement of cell-matrix and cell-cell interactions in the process of metastasis has pointed to the complexity of the malignant phenotype. Studies of fibronectin, vitronectin, and laminin receptor functions indicate that inhibition of tumor cell adhesion results in less aggressive invasive behavior. Data suggest that both decreased tumor cell attachment through modulation of integrin receptor function and enhanced attachment via CD44 and VLA-4/VCAM systems are important in formation of tumor metastasis. Although these data appear to be somewhat contradictory, they most likely address the differences between growth control (tumorigenicity), which may be modulated by matrix receptor function, and the process of invasion, which is necessarily associated with the ability to attach to the extracellular matrix in order to arrest, extravasate, and migrate. The ability to invade may require an intermediate expression of adhesive capability, with too little resulting in the inability of a circulating tumor cell to arrest and invade a secondary site. At the other end of the spectrum, cells that are extremely adherent, either to the extracellular matrix or to each other, may be unable to begin the metastatic process and migrate from the primary tumor. The resolution to these conflicts lies in defining

the specific control mechanisms through which cellular adhesion and motility are coupled to produce the invasive phenotype.

PROTEOYLSIS OF THE EXTRACELLULAR MATRIX DURING TUMOR INVASION

Although proteolysis and migration through tissue barriers are normal cell functions in specific physiologic circumstances, clearly a general aspect of malignant neoplasia includes a shift toward sustained invasive capacity. For invasion to take place, cyclic attachment to and subsequent release from matrix components must occur in a directed and controlled manner. This implies that proteolysis, although enhanced in tumor cells, is still tightly regulated in a temporal and spatial fashion with respect to cell attachment and migration. Proteolytic activity is the balance between the local concentration of activated enzymes and their endogenous inhibitors.

Positive correlation between tumor aggressiveness and protease levels has been documented for all four classes of proteases; thiol-, seryl- aspartyl- and metallo-proteases. All of the enzymes implicated in this association have been identified in normal cells. The association of these proteases with the invasive process is through inappropriate overexpression in the tumor tissue, either by the tumor cells, host cells intermixed or immediately adjacent to the invasion front, or both. In addition to protease enhancement, augmented heparanase activity has also been associated with malignant invasion (Nakajima et al 1990, 1987).

Over the last five years a significant body of evidence has accumulated that directly implicates members of the matrix metalloproteinase (MMP) family in tumor invasion and metastasis. This enzyme family currently includes eight members. The criteria used to define members of the MMP family include zinc metal-atom dependency, secretion as a zymogen, in vitro activation of the proenzyme by organomercurial reagents, auto-proteolytic removal of the N-terminus following activation, and inhibition by a specific class of biological inhibitors referred to as the tissue inhibitors of metalloproteinases (TIMPs). Profragment removal following organomercurial activation is the result of intramolecular endoproteolytic activity. Thus proenzyme activation must occur prior to removal of the profragment. Profragment loss is therefore indicative of, but not equivalent to, proenzyme activation.

Subgroups within the MMP family have traditionally been defined based on substrate specificity. This family of endopeptidolytic enzymes is now subdivided into three general classes: interstitial collagenases, stromelysins, and gelatinases (type IV collagenases). The members of the MMP gene family, their domain structure, substrate specificity, and activation mecha-

nisms have been the subject of several excellent reviews to which the reader is referred (Matrisian 1992; Nagase et al 1991; Woessner 1991).

MATRIX METALLOPROTEINASES IN TUMOR INVASION

Evidence for the role of MMP enzymes in tumor invasion and metastasis comes from a variety of studies. These include many in vitro studies of murine and human tumor cell lines that transcribe, synthesize, and secrete MMP enzymes (Lyons et al 1991; Matrisian et al 1991; Sato et al 1992; Templeton et al 1990). In fact, several of the members of the MMP family were first identified, purified, and cloned from tumor cell lines. Certainly this is true of the type IV collagenases, now known as gelatinase A and gelatinase B, transin (the murine homologue of human stromelysin) as well as stromelysin-2 and matrilysin (formerly PUMP-1). These enzymes are also synthesized and secreted by normal cells under conditions that may be associated with physiologic tissue remodeling. The difference between enzyme production under physiologic and neoplastic conditions may be that in tumor cells the enzymes may be constitutively overexpressed or induced by autocrine growth factor stimulation. Tumor cells may also be unresponsive to signals from host cells and matrix that would down-regulate MMP expression of normal cells. While overexpression in tumor cells was an aid to their identification, isolation, and characterization, it may have led to the unfounded presumption that some MMPs are tumor cell-specific.

Studies have clearly demonstrated a positive correlation between MMP expression, invasive behavior, and metastatic potential in animal models (Bonfil et al 1989; Powell et al 1993; Sreenath et al 1992). Studies have also shown that growth factors can dramatically modulate MMP expression (Brown et al 1990; Matrisian 1992; Ogata et al 1992b, Weinberg et al 1990). For example, epidermal growth factor, transforming growth factor alpha, and platelet-derived growth factor have all been shown to upregulate transcription of MMPs, most notably interstitial collagenase and stromelysin. Autocrine growth factor stimulation, in addition to supporting autonomous growth necessary for clonal dominance (Kerbel 1992), may stimulate MMP production and thereby support the metastatic phenotype at several levels.

Extracellular matrix components, cell-matrix interactions, and the pericellular environment are also important determinants of MMP production. Stimulation of the $\alpha_v\beta_3$ integrin receptor (vitronectin receptor) enhanced gelatinase A production and stimulated melanoma cell invasion (Seftor et al 1992). Laminin A chain peptide fragments induced gelatinase A production, local invasiveness and augmented metastasis formation (Fridman et al 1991; Fridman et al 1992b; Sweeney et al 1991). Acidic culture environment

and tumor necrosis have been shown to enhance production of MMP activities, most notably gelatinases A and B (Bonfil et al 1992; Kato et al 1992).

Host-tumor interactions may also greatly influence protease production. Indeed the MMP profile and metastatic competence of KM12SM human colorectal tumor cells is influenced by the site of implantation (Nakajima et al 1990). Subcutaneous implantation of tumor cells did not result in visceral metastases, whereas cecal implantation led to lymph node and hepatic metastases. The tumor explants from the cecum wall demonstrated an increase in the active forms of gelatinase A, as well as significant elevation of type IV collagenolytic and heparanase activities.

CORRELATION OF MATRIX METALLOPROTEINASE EXPRESSION WITH INVASIVE BEHAVIOR OF HUMAN TUMORS

Researchers have begun to examine MMPs in human tumor tissues and serum from cancer patients. These efforts have included immunoperoxidase staining (IPS) of tissue sections for localization of MMPs in human tumor tissues, Northern blot analysis of MMP transcripts in RNA samples extracted from human tumor samples, in situ hybridization (ISH) studies of MMP transcripts, and measurement of MMP levels in the body fluids of cancer patients.

Interstitial collagenase, which degrades the triple helical domains of the fibrillar collagens (types I, II, III, and X), is augmented in many human tumors. The level of proteolytic activity against soluble type I collagen demonstrated a statistically significant correlation with the degree of histologic differentiation in human colorectal tumors (van der Stappen et al 1990). IPS studies revealed enhanced staining for interstitial collagenase in the stromal cells and collagen fibers immediately adjacent to the malignant nests of colorectal tumor cells (Hewitt et al 1991). Little evidence for collagenase staining of normal, benign, or malignant epithelium was observed. Normal colorectal tissues and adenomas were negative for interstitial collagenase staining. Elevated interstitial collagenase transcripts have been observed in 40% of primary pulmonary malignancies, but not in samples from adjacent normal lung tissue (Urbanski et al 1992). ISH demonstrated elevated mRNA transcripts for interstitial collagenase in squamous cell carcinomas of the head and neck that localized to the stromal fibroblasts immediately adjacent to the malignant tumor masses (Gray et al 1992), thus confirming earlier reports (Muller et al 1991; Polette et al 1991).

Experimental results consistently localize interstitial collagenase production to the stromal fibroblasts immediately adjacent to the site of tumor

invasion, which suggests that invasive tumor epithelial release is a stimulus for induction of fibroblast synthesis of this enzyme. A tumor cell-derived collagenase stimulatory factor has been characterized and partially sequenced (Nabeshima et al 1991). This sequence data shows no homology with known growth factors, motility factors, or collagenase stimulatory agents. Originally purified from a human lung carcinoma cell line, this stimulatory factor is released into tumor cell media and is also associated with the tumor cell membranes. This tumor cell-derived collagenase stimulatory agent also stimulates the synthesis and secretion of gelatinase A and stromelysin from human fibroblasts in culture (Karaoka et al 1993).

Members of the stromelysin group of MMP enzymes include stromelysins 1, 2, and 3, as well as matrilysin. Matrilysin lacks a C-terminal domain and is the smallest member of the MMP family. These enzymes have a fairly broad range of protease activity-degrading glycoproteins such as laminin and fibronectin, proteoglycans, and nonhelical domains of type IV collagen. Matrilysin can cleave urokinase to separate the catalytic and receptor-binding domains, which suggests that matrilysin activity may be important in regulating functional activity of this plasminogen activator (Marcotte et al 1992).

Expression of stromelysins 1 and 2 has been studied in carcinomas of the head and neck. In these studies, high levels of stromelysin mRNA transcripts were correlated with increased local invasiveness (Muller et al 1991). Transcripts were localized principally to the fibroblasts of the tumor stroma adjacent to areas of basement membrane disruption (Polette et al 1991). ISH studies have also shown that matrilysin, but not stromelysin 1 or stromelysin 2, are overexpressed in human gastric and colonic carcinomas (McDonnell 1991).

Matrilysin transcripts were also observed in 14 out of 18 RNA samples isolated from human prostate adenocarcinomas and in 3 out of 11 normal prostate biopsy samples (Pajouh et al 1991). ISH localization showed that matrilysin was expressed in the epithelial cells of the primary prostate adenocarcinoma and in some foci of epithelial dysplasia, but not in the stroma.

The most recently described member of the stromelysin subgroup, stromelysin 3, was discovered through its association with human breast cancer progression (Basset et al 1990). The endoproteolytic activity and substrate specificity of stromelysin 3 have yet to be defined. Like other stromelysins, stromelysin 3 expression was localized to the stromal cells surrounding invasive human breast carcinomas. Stromelysin 3 expression has also been studied in squamous cell carcinomas of the head and neck (Muller et al 1993), basal cell carcinomas (Wolf et al 1992), and primary pulmonary carcinomas (Urbanski et al 1992). In all of these studies there

is a consistent association of stromelysin 3 expression with the stromal fibroblasts adjacent to the malignant epithelium. In the head and neck tumors, stromelysin 3 expression correlated with the degree of local invasiveness (Muller et al 1993). Although stromelysin 3 shows a consistent association with the invasive and malignant potential of human tumors, it is by no means specific for this process. Recent demonstration of stromelysin 3 in cutaneous wound healing (Wolf et al 1992) and apoptotic process of postlactating mammary gland involution (Lefebvre et al 1992) have led to the proposal that stromelysin 3 expression corresponds to a normal fibroblast response that is exacerbated in invasive carcinomas (Wolf et al 1992). This is consistent with our original proposal that tumor-associated MMP overexpression is a dysregulation of normal protease systems.

The third group of enzymes of the MMP gene family are the gelatinases A and B, formally referred to as type IV collagenases. These enzymes rapidly degrade denatured collagens (gelatin), as well as a number of native collagen types that contain helical disruptions. The 72-kd gelatinase A was first described through its ability to degrade pepsinized, triple-helical type IV collagen and its association with tumor cell invasion of the basement membrane. The two gelatinases arise from separate mRNA transcripts (Collier et al 1988; Wilhelm et al 1989) and are distinct from other members of the MMPs in that they possess a unique region immediately adjacent to the putative metal-binding domain that is homologous to the gelatin-binding domain of fibronectin and may function in substrate binding. Gelatinase A and B also differ from other members of the family by their ability to interact, as latent proenzymes, with the endogenous inhibitors of these enzymes, the TIMPs (Goldberg et al 1989; Stetler-Stevenson et al 1989; Wilhelm et al 1989). These proenzyme-inhibitor complexes are specific with progelatinase A binding TIMP-2 and progelatinase B binding TIMP-1.

Evidence for the expression of gelatinase A in human tumors is abundant. Most breast, colonic, and gastric adenocarcinomas are immunoreactive for 72-kd gelatinase A, whereas benign proliferative disorders of these tissues are negative (Campo et al 1992a,b; Clavel et al 1992; D'Errico et al 1991; Levy et al 1991; Monteagudo et al 1990; Ohori et al 1992; Pyke et al 1992). IPS for gelatinase A in malignant breast epithelium was reportedly more frequent (16 out of 22 cases) than either stromelysin 1 (12 cases) or interstitial collagenase (9 cases) (Clavel et al 1992). Strong immunostaining of the malignant epithelium both in invasive and preinvasive prostatic carcinoma has been reported (Boag & Young 1993). Recent studies of serous tumors of the ovary were unable to detect gelatinase A in benign cysts, yet invasive growths were positive (Campo et al 1992b). IPS studies of the 72-kd gelatinase A enzyme also has been found to correlate with tumor grade in neoplastic thyroid (Campo et al 1992a). However, gelatinase

A was also detected in benign disorders in which the tissue was undergoing remodeling and repair. Gelatinase A has also been demonstrated in the sclerosing and mucinous variants of brochioalveolar carcinoma, possibly contributing to the poorer prognoses of these subgroups (Ohori et al 1992). In summary, IPS studies have shown expression of gelatinase A in many types of human tumors, and this expression is usually limited to the malignant epithelial cells.

Elevated gelatinase mRNA transcripts have also been identified in RNA extracted from primary human pulmonary carcinomas (Urbanski et al 1992), colon carcinomas (Levy et al 1991), and primary breast cancers (Basset et al 1990). Gelatinase B was found in five of nine pulmonary tumors. Although gelatinase A transcripts were expressed in the majority of carcinomas, they were also occasionally present in normal uninvolved lung tissue. These observations are similar to those previously reported for the gelatinases when studied in primary breast cancers (Basset et al 1990).

Cellular localization of gelatinase production has been performed by ISH in a number of tumor systems. Signals for both gelatinases were distributed over the neoplastic epithelium as well as stromal elements of the primary pulmonary tumors (Urbanski et al 1992). ISH detected gelatinases A and B in infiltrating basal and squamous cell carcinomas (Pyke et al 1992) that localized to the stromal fibroblasts adjacent to the sites of tumor invasion. Gelatinase B has also been localized to eosinophils infiltrating the dermis in response to invasive basal cell carcinoma. ISH studies of human colon carcinomas demonstrate gelatinase A localized primarily to the stromal fibroblasts immediately adjacent to sites of invasion (Poulsom et al 1992). This stromal localization is distinct from the predominant IPS localization of gelatinase A in the malignant epithelium performed in the same report.

This discrepancy between the stromal localization of gelatinase A mRNA transcripts by ISH and the predominant IPS reactivity in the malignant epithelial cells has led some investigators to propose that the stromal fibroblasts at the invasive front are resposible for the bulk of gelatinase A expression associated with tumor invasion. It has also been suggested that this discrepancy is evidence for a cell-surface receptor on tumor cells for gelatinase A enzyme. Indeed, such a surface receptor for gelatinase A has been identified on human breast cancer cell lines MCF7 and MDA MB231, and preliminary characterization has been reported (Emonard et al 1992). However, alternative explanations for this discrepancy are equally likely. Given the diffuse IPS staining pattern for gelatinase A in many human tumors, it seems rather unlikely that stromal fibroblasts at the invasive front are responsible for producing enzyme that is detected at the center of the tumor mass. Thus it is unlikely that the stromal fibroblasts are the sole

source for this enzyme. Another possible explanation for the discrepancy between ISH and IPS results is the relative sensitivity of these methods. Tumor cells may have low constitutive levels of gelatinse A transcriptional activity, whereas stromal fibroblasts may have a strong, highly induced, but short, temporal burst of gelatinase A transcriptional activity in response to stimuli at the invasion front. Fibroblasts that are reactive to the invasive stimulus may have high gelatinase A transcriptional activity, but low translational activity that results in low cytoplasmic stores of this enzyme. This question could be addressed by in vitro co-culture experiments and/or comparison of transcriptional and translational activity in tumor cells vs activated fibroblasts.

The correlative studies of MMP expression in human tumors suggest that these proteases are important in the biology of human tumors and may make a significant contribution to the invasive phenotype of such tumors. The picture obtained from these studies, however, is complex and still incomplete. Clearly no single member of the MMP family is responsible for the invasive phenotype of all human malignancies. Invasive tumors apparently utilize a number of different strategies for breakdown and crossing of matrix barriers as reflected in the heterogeneity of the MMP enzymes and other proteases expressed during this process.

Correlation of metalloproteinase expression with local recurrence, lymph node metastasis, distant metastasis, and/or patient survival allows assessment of the utility of these measurements as diagnostic or prognostic markers. Overexpression of gelatinase A has been correlated with local recurrence in human breast cancer (Daidone et al 1991) as well as with an increased frequency of lymph node metastasis in human gastric carcinoma (Otani 1990). Both gelatinase A and stromelysin expression have been correlated with lymph node metastasis and vascular invasion in human esophageal carcinoma (Shima et al 1992). IPS studies of gelatinase A in 46 patients with squamous carcinoma of the head and neck found high level expression in 77% of patients with lymph node metastases, but only 25% in patients without lymph node metastases (Kusukawa et al 1993). Expression of gelatinase A appears to be a useful marker for evaluating the malignant potential of individuals with squamous cancers of the oral cavity.

Gelatinase A and B enzymes are normal components of human plasma, and recent studies have begun to evaluate the diagnostic utility of measurement of these enzyme levels in plasma, serum, and other body fluids from cancer patients. Measurement of total serum gelatinase A levels using a substrate-capture assay correlated tumor burden in patients with primary pulmonary malignancies and may be a useful indicator of response to therapy (Garbisa et al 1992). Plasma levels of gelatinase A were not elevated in

patients with breast or colon cancer (Zucker et al 1992), but gelatinase B levels were elevated in patients with cancer of the colon or breast, with no significant elevation in plasma levels of this enzyme in patients with lung tumors, genitourinary cancer, or leukemia/lymphomas (Zucker 1993). Significant elevations of the plasma gelatinase B levels were also observed during pregnancy. Concentrations of the gelatinases A and B in the pleural fluids of a variety of patients were independent of the serum concentration of these enzymes, but were not specific in discriminating nonmalignant from malignant pleural effusions (Hurewitz et al 1992). As a group, patients with transitional cell cancer of the bladder showed statistically significant elevation of gelatinase A and fragments of this enzyme in their urine (Margulies et al 1992).

PROENZYME ACTIVATION: An Important Control Point in Development of the Invasive Phenotype

All of the studies reviewed to this point have determined levels of total MMP enzyme protein or steady-state levels of MMP mRNA transcripts. However, it is known that all members of the MMP enzyme family are secreted in zymogen form and must be activated in the extracellular milieu prior to obtaining extracellular matrix degrading activity. This suggests that measurement of active enzyme species may be more informative with respect to the state of matrix turnover and possibly of more diagnostic or prognostic utility. Investigators have utilized quantitative gelatin zymography to assess the contribution of active gelatinase species to the invasive phenotype of human breast and non-small cell lung cancer (Brown et al 1993a,b; Davies et al 1993). These studies demonstrate that although many tumor tissues express both gelatinase A and B in zymogen form, only small amounts of the activated form of the 92-kd gelatinase B enzyme could be detected in some tumors. The fraction of total gelatinase A enzyme present as the 62-kd activated form of the enzyme was statistically elevated in malignant disease, and a higher proportion of this active enzyme species was detected in higher grade tumors (Davies et al 1993). Detection of activated forms of gelatinase A enzyme occurs more frequently in invasive human breast tumors than gelatinase B, which implies that activation of the gelatinase A is a feature of the invasive phenotype in breast cancer patients, whereas activation of gelatinase B is not. These studies emphasize that these enzymes are secreted as latent proenzymes and require activation prior to obtaining proteolytic activity. Overproduction of proenzyme species is necessary but not sufficient for the development of the invasive phenotype, and proenzyme activation is an important step in the acquisition of the invasive phenotype.

CELLULAR MECHANISM FOR ACTIVATION OF GELATINASE A (MMP-2)

Gelatinases A and B, like other members of the collagenase family, are secreted as latent proenzymes and must be activated extracellularly. But unlike other proenzymes in this family, progelatinase A and B may be complexed with endogenous inhibitors known as tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2). Progelatinase A selectively binds TIMP-2 and progelatinase B binds TIMP-1 preferentially (Goldberg et al 1989; Stetler-Stevenson et al 1989; Wilhelm et al 1989). The activation of these enzymes and/or enzyme-inhibitor complexes therefore constitutes an important, possibly unique, level of regulation for these enzymes. Recent in vitro studies of the mechanism of activation and domain structure of these enzymes has yielded new insights into the molecular basis of proenzyme latency and proenzyme-inhibitor interactions (Fridman et al 1992a; Howard & Banda 1991; Howard et al 1991; Kleiner et al 1992, 1993; Murphy et al 1992b).

Progelatinases A and B, like all matrix metalloproteinases with the exception of stromelysin-3, can be activated in vitro by a variety of agents including organomercurials, chaotropic agents, and other proteases. Proteases, specifically plasmin, have been shown to be responsible for the activation of both interstitial procollagenase and prostromelysin-1 in co-cultures of keratinocytes and dermal fibroblasts (He et al 1989; Murphy et al 1992a). The activation of the 92-kd gelatinase B has also been studied in vitro. Like interstitial collagenase and stromelysin-1, the 92-kd gelatinase can be activated by other proteinases such as trypsin and plasmin. Recent studies have shown that gelatinase B can be activated by stromelysin and that formation of TIMP-1/progelatinase B complex can modulate this activation (Goldberg et al 1992; Murphy et al 1992a; Ogata et al 1992a). Both plasmin and cathepsin B have been shown to activate prostromelysin, which suggests that either may initiate a cascade of MMP activation, similar to that of the blood clotting mechanism, as has been proposed by Matrisian (1992) and Murphy et al (1992a). However, plasmin and a variety of other proteases do not activate the 72-kd gelatinase A (Okada et al 1990).

Activation of gelatinase A or the gelatinase A/TIMP-2 complex does not appear to occur in the soluble phase as part of a proteolytic cascade similar to other members of the matrix metalloproteinase family (Brown et al 1990, 1993c; Murphy et al 1992b, Okada et al 1990; Ward et al 1991). Activation of this enzyme requires interaction with the cell surface, and this cellular activation mechanism requires the presence of an intact C-terminal domain on the gelatinase A (Murphy et al 1992b). Treatment of HT-1080

fibrosarcoma cells with phorbol ester or treatment of fibroblasts with concanavalin A induces processing of the 72-kd gelatinase A to a 62-kd activated form (Brown et al 1993c; Overall & Sodek 1990). The activity responsible for processing the latent complex to active enzyme was found to be confined to the cell monolayer and was not secreted into the soluble fraction. The existence of a plasmin-independent, cell-surface mechanism for the specific activation of a single collagenase family enzyme has important physiologic implications. Such a mechanism would give tight cellular control over matrix degradation by this enzyme and would limit proteolysis to the immediate vicinity of the cell surface. With respect to tumor invasion and metastasis, it might allow a tumor cell that expresses the activator, but not gelatinase A, to activate and utilize exogenous gelatinase A produced by stromal cells in response to the local presence of invading tumor cells. The identification of the molecular species responsible for the cellular activation of the 72-kd gelatinase A is likely to yield an important new series of target molecules in the development of treatments for invasive and degradative diseases.

TISSUE INHIBITORS OF METALLOPROTEINASES DEFINE THE FUNCTIONAL ROLE OF MMPS IN CELL INVASION

Activation of proenzyme is an important control point for development of the invasive phenotype, but activation alone may not be sufficient to obtain the invasive phenotype if this occurs in the presence of excess tissue inhibitors of matrix metalloproteinases (TIMPs), the endogenous and ubiquitous inhibitors of MMPs. Thus it is the balance of active enzyme and TIMP that will determine if local matrix degradation occurs. The numerous correlative studies outlined above suggest that overexpression of matrix metalloproteinase proenzymes and subsequent activation is the mechanism by which tumor cells achieve a balance in favor of proteolysis. Recent direct evidence, using exogenous TIMPs to alter this protease/inhibitor balance, has been obtained and establishes an effector role for MMP activities in tumor cell invasion.

TIMP-1, the first member of the TIMP family to be identified (Carmichael et al 1986; Cawston et al 1981; Welgus & Stricklin 1983) is a glycoprotein with an apparent molecular mass of 28.5 kd. TIMP-1 forms a complex of 1:1 stoichiometry with activated interstitial collagenase, activated stromelysin, and both activated and progelatinase B. The *Timp-1* gene has been mapped to the p11 region of the human X chromosome (Mahtani & Willard 1988; Willard et al 1989).

TIMP-2 is a 21 kd, non-glycosylated protein that shows 37% amino acid

identity and 65.6% overall homology to TIMP-1, yet the proteins are immunologically distinct (Boone et al 1990; DeClerck et al 1989, 1991, 1992a; Goldberg et al 1989; Stetler-Stevenson et al 1990). The *Timp-2* gene has been localized to chromosome 11 in the mouse and human chromosome 17q25 (DeClerck et al 1992b, Stetler-Stevenson et al 1992). Northern blot analysis has revealed the existence of two *timp-2* transcripts of 3.5 and 1.0 kb. cDNA probes to *timp-2* are specific and do not detect the single 0.9 kb *timp-1* mRNA species. *timp-1* and *timp-2* expression are regulated independently. The pattern of expression and specific roles for these inhibitors in vivo remain areas of active investigation.

Native or recombinant TIMP-1 has been shown to inhibit in vitro invasion of human amniotic membranes (Mignatti & Robbins 1986; Schultz et al 1988) and in vivo metastasis in animal models (Alvarez et al 1990; Schultz et al 1988). Furthermore, transfection of antisense TIMP RNA into mouse 3T3 cells, which down-regulates TIMP-1 expression, enhances their ability to invade human amniotic membranes and to form metastatic tumors in athymic mice (Khokha et al 1989). TIMP-2 has been shown to successfully inhibit in vitro tumor cell invasion of extracellular matrices (Albini et al 1991; DeClerck et al 1991). Overexpression of TIMP-2 in invasive and metastatic *ras*-transformed rat embryo fibroblasts resulted in suppression of the ability of these cells to form lung colonies following intravenous injection in nude mice (DeClerck et al 1992a). Increased TIMP-2 levels significantly reduced the in vivo growth rate and invasive character of tumors following subcutaneous injection of these transfected cells. Recent studies have shown an inverse correlation between TIMP-2 expression and invasive potential of the HEp-3 human epidermoid carcinoma cell lines following extended in vitro culture and passage (Testa 1992). This suggests that in some tumors enhanced TIMP-2 expression may alter the balance of activated MMP and inhibitor, thus inhibiting matrix proteolysis.

Recent studies also suggest that TIMPs are capable of inhibiting angiogenesis, which has many functional aspects similar to the process of tumor cell invasion. Mignatti et al reported that TIMP-1 inhibited in vitro endothelial cell invasion of human amniotic membranes (Mignatti & Robbins 1986). Moses et al presented data showing that cartilage-derived inhibitor (CDI), a TIMP-related protein isolated from bovine articular cartilage, can block angiogenesis and also inhibit endothelial cell proliferation (Moses & Langer 1991; Moses et al 1990). Both TIMP-1 and TIMP-2 have been shown to inhibit chick yolk sac vessel morphogenesis in response to polyamines (Takigawa et al 1990). The balance of TIMPs and gelatinase activity appears to be an early and critical determinant of endothelial morphogenesis and tube formation during in vitro growth on matrigel matrix (Schnapper et al 1993). Collectively, these data support a role for colla-

genolytic activity in at least two functional processes contributing to metastasis. That is, collagenases are involved in tumor cell invasion as well as neovascularization, upon which solid tumor growth is dependent. These are important considerations in the design of proteolytic inhibitors for potential use as therapeutic agents.

THE INVASIVE PHENOTYPE IS A BALANCE OF ACTIVE PROTEASES AND THEIR INHIBITORS

The critical nature of the balance between active proteases and their inhibitors for the success of the invasive phenotype has been demonstrated in two experimental systems. The first system is an in vitro model of tumor cell invasion using amniotic membranes. Researchers have demonstrated a bimodal relationship between invasion of amniotic membranes and plasminogen activator activity (Tsuboi & Rifkin 1990). In these experiments it was shown that the in vitro invasive behavior of Bowes' melanoma cells, which produce large amounts of tissue plasminogen activator, and HT1080 fibrosarcoma cells, which produce large amounts of urokinase-type plasminogen activator, is not blocked but enhanced by the addition of plasmin inhibitors or anti-plasmin antibodies. Conversely, the invasive capacity of cells that produce low levels of these proteases was blocked by the addition of inhibitors. Successful cellular invasion requires a balance of proteases and protease inhibitors. Protease activity in excess of the optimal level may result in uncontrolled local matrix degradation and interrupt cell-matrix interactions necessary for invasion.

The second system is a model for angiogenesis. As mentioned previously, angiogenesis shares many functional similarities with tumor metastasis including the requirement for expression of the invasive phenotype and associated proteolytic activity. It has been shown that agents that induce angiogenesis, such as bFGF, will induce endothelial expression of both urokinase (uPA) and plasminogen-activator inhibitor-1 (PAI-1), with the balance slightly in favor of uPA (Pepper et al 1990). In these assays, a balance in favor of protease inhibition resulted in the formation of solid cords of endothelial cells rather than tubes. Montesano and co-workers (1990) studied the formation of angiomas (benign endothelial tumors) in fibrin gels. The angioma-forming cell lines produced hemangioma-like cystic structures in the fibrin gels. Addition of exogenous serine protease inhibitors resulted in the formation of endothelial cords instead of cystic structures. Thus protease/antiprotease balance can alter the morphology of the capillary tube with excessive proteolysis resulting in sac-like noninvasive structures.

THE ROLE OF TUMOR CELL MOTILITY IN INVASION AND METASTASIS

Following organogenesis and tissue differentiation, the migration of epithelial cells is a very rare event. This is in contrast with the active cell motility that is a necessary feature of invasive carcinoma cells. Active cell motility, coupled with matrix proteolysis, is required for the penetration of extracellular matrices during expression of the invasive phenotype. Invasive tumor cells also exhibit directional motility during intravasation and extravasation. The importance of tumor cell motility to invasion and metastasis has been appreciated for more than 40 years (Coman 1953; Enterline & Coman 1950). Tumor cell lines known to be more highly invasive and metastatic showed a higher degree of motility than their low metastatic counterparts, when parameters such as pseudopod extension, membrane ruffling, and vectorial translation were measured.

A variety of agents appear to stimulate motile responses in tumor cells in vitro including host-derived scatter factors (Rosen et al 1990; Weidner et al 1990), growth factors (Aznavoorian et al 1990; Jouanneau et al 1991; Kahan & Kramp 1987; Stracke et al 1988), components of the extracellular matrix (Lester et al 1989, 1991; McCarthy & Furcht 1984; McCarthy et al 1986; Taraboletti et al 1987; Yusa et al 1989), hyaluronan (Turley 1992; Turley et al 1991), and tumor-secreted factors (Atnip et al 1987; Liotta et al 1986; Ohnishi et al 1990; Seiki et al 1991; Siletti et al 1992; Stracke et al 1992). Motility stimulated by each of these factors can be either random in nature (chemokinesis) or directed (chemotaxis). Chemotaxis is defined as directional migration of cells in response to concentration gradients of soluble factors. Tumor cells also migrate in a directional manner, in the absence of soluble attractant, towards substratum-bound, insoluble ECM proteins (haptotaxis) (Aznavoorian et al 1990; McCarthy et al 1985). These numerous stimuli could provide tumor cells with multiple opportunities for transiting different microenvironments during the metastatic process.

A family of tumor cell-derived motility-inducing cytokines has been discovered and termed autocrine motility factors (AMF). Studies with cultured human A2058 melanoma cells demonstrated that these cells produced an attractant material in serum-free media that was approximately 60 kd in molecular mass (Liotta et al 1986). This AMF stimulated both random (chemokinetic) and directed (chemotactic) motility in the same cells in which it was synthesized. The discovery of AMF led to the proposal that cells in the primary tumor presumably secrete AMF until the concentration rises enough to stimulate motility via receptors on the responding cells.

AMF stimulates chemotaxis in A2058 melanoma cells through a pertussis toxin- (PT) sensitive receptor (Stracke et al 1987), which results in directed pseudopodial protrusion (Guirguis et al 1987). However, a variety of agents that affect adenylate cyclase have no effect on AMF-stimulated motility, thus indicating that cAMP is not the necessary second messenger (Stracke et al 1987). As with phagocyte chemotaxis (Garcia-Castro et al 1983), methylation of phospholipids appears to be a component of the biochemical cascade of activation (Liotta et al 1986). AMF specifically induces enhanced motility in a variety of tumor cells, but fails to stimulate leukocyte migration (Liotta et al 1986). Other tumor-derived factors with a similar molecular mass have subsequently been reported and purified by several investigators (Atnip et al 1987; Ohnishi et al 1990; Schor et al 1988; Siletti et al 1992).

A potent new motility stimulator of approximately 120 kd was isolated from A2058 cell-conditioned medium, purified to homogeneity (Stracke et al 1992), and was termed autotaxin (ATX). ATX is a basic glycoprotein (pI ~7.7), active in the picomolar range, that stimulates both chemotactic and chemokinetic responses in A2058 cells. Identical to AMF, cells pre-treated with pertussis toxin lack a motile response to purified ATX. Direct sequencing of ATX peptides has shown no sequence homologies with known growth or motility factors and may represent a new member of the AMF family.

The use of ECM macromolecules as attractants in motility assays illustrates the fundamental differences between chemotaxis and haptotaxis with respect to post-receptor signal transduction events. A2058 melanoma cells are chemotactic and haptotactic on gradients of ECM proteins, such as laminin, fibronectin, type IV collagen (Aznavoorian et al 1990), and thrombospondin (Taraboletti et al 1987). When cells are pre-treated with pertussis toxin, the chemotactic response to laminin is diminished, and the response to type IV collagen is abolished (Aznavoorian et al 1990). In contrast, haptotaxis to these same proteins is completely insensitive to pertussis toxin. In the case of fibronectin, neither chemotaxis nor haptotaxis is affected by PT. One explanation of these data is that chemotaxis and haptotaxis to the same ECM protein are mediated by distinct cell-surface receptors that recognize different domains of the large multi-domain matrix proteins. Chemotaxis and haptotaxis receptors apparently generate motility signals through different transduction mechanisms. Studies with thrombospondin (Taraboletti et al 1987) identified distinct chemotaxis- and haptotaxis-promoting domains on this molecule. Differential inhibition of each type of migration with specific antibodies and/or cell-binding peptides prevented cell interaction with the relevant regions. Both haptotatic and chemotactic responses may be relevant

to the metastatic phenotype. During the initial stages of metastasis, haptotactic migration over insoluble matrix proteins may be the more significant response. Later, partially degraded matrix proteins that result from proteolytic processing of the matrix could be involved in chemotactic responses that may then dominate the migratory phenotype.

Insulin-like growth factors and insulin stimulate a pertussis toxin-insensitive chemotactic response in A2058 cells (Stracke et al 1988). This response is strongest to IGF-I and appears to activate the cells through a type I IGF receptor (Stracke 1989). Both insulin and IGF-I have been implicated as necessary growth factors for culture of primary human melanoma cells (Rodeck et al 1987). This requirement suggests that IGF-I may serve as a kind of homing factor for tumor cells that have reached the vasculature. Secretion of IGF-1 could facilitate the extravasation of tumor cells into a secondary site that provides the necessary microenvironment for continued growth of the metastatic foci.

The detailed molecular mechanisms that stimulate cells to migrate directionally are incompletely understood. Morphological studies demonstrate that tumor cells, like leukocytes, exhibit amoeboid movement characterized by pseudopod extension (Hosaka et al 1979; Mohler et al 1987). This kind of motility requires the coordinated action of distinct steps including cellular protrusion at the leading edge, new adhesion formation, and release of old adhesions at the trailing edge, which result in the asymmetric morphology of motile cells observed on two-dimensional substrates. It is generally agreed that the machinery for cell locomotion in eukaryotic cells resides in the peripheral cytoplasm, or cell cortex, which consists of a network of polymerized, cross-linked actin filaments (Condeelis et al 1992; Cunningham 1992; Stossel 1990). For pseudopod protrusion and cell locomotion to occur, this network must be reversibly disassembled, or solated, to allow protrusion, then re-assembled to stabilize the resulting extension. The precise manner in which this is accomplished, as well as its linkage to a localized, receptor-mediated chemotactic stimulus, is still not fully understood, although studies with neutrophils and with the lower eukaryote *Dictyostelium discoideum* have provided some insights that may be applicable to tumor cells. Models proposed by Condeelis et al (1992) and Stossel (1990) envision the localized polymerization and cross-linking of actin filaments at the site of a chemotactic stimulus, which result in the directional protrusion of a pseudopod. Both models speculate that the signals generated by activated chemotactic receptors, and the resulting second messengers, regulate proteins that sever, cap, and cross-link actin filaments to generate pseudopods. The model based on studies with leukocytes links actin disassembly and re-assembly with the activation of the phos-

phatidylinositol (PI) cycle (Lester & McCarthy 1992). Thus in leukocytes, chemotactic receptor stimulation leads to activation of the PI cycle, actin polymerization, and motility; events that are mediated by a pertussis toxin-sensitive G protein (Goldman et al 1985; Spangrude et al 1985). In tumor cells, several lines of evidence also implicate the involvement of PT-sensitive G proteins in motility and invasion (Aznavoorian et al 1990; Lester et al 1989, 1991; Roos & Van De Pavert 1987; Stracke et al 1987, 1992). Indeed, using specific antibodies and oligonucleotide probes, G_ia_2 has been identified as the PT-sensitive G protein uniquely abundant in highly metastatic murine melanomas (Lester et al 1989, 1991), which leads to the speculation that this G protein contributes to the regulation of motility in highly invasive and metastatic tumor cells. However, as stated above, chemotactic migration of the A2058 human melanoma cell line to IGF-I and fibronectin (Stracke et al 1988) and haptotactic migration to laminin, fibronectin, and type IV collagen (Aznavoorian et al 1990) are insensitive to pertussis toxin treatment. Therefore, care must be taken not to implicate G_ia_2 as a general regulator of motility in all tumor cells. It appears, instead, that tumor cells are equipped to respond in a variety of ways to a diverse array of motility stimuli. This would obviously give tumor cells a great deal of flexibility as they encounter different microenvironments during invasion and metastasis, and would impart a selective advantage over cells that are less adaptable.

SUMMARY

Recent findings have produced great strides in developing an understanding of the molecular events involved in processes necessary for tumor cell invasion and subsequent metastasis formation. This information has been useful in developing new targets for therapeutic intervention such as disruption of tumor cell attachment by peptide analogues of cell adhesion molecules and the use of protease inhibitors to limit extracellular matrix proteolysis required for tumor cell invasion. Future efforts must focus on how the events of cell attachment, matrix proteolysis, and cell migration are controlled and integrated. This requires a better understanding of the transcriptional controls and cell signaling mechanisms that are involved in these events. Preliminary findings suggest that cell-matrix interactions influence gene expression and that the protease inhibitor balance can greatly influence cell-matrix interactions. Therefore it appears that all three steps in the invasive process are linked and interdependent. While this complicates the study of these processes, it is our belief that understanding this interdependence is critical for further development of metastasis research.

Literature Cited

Albelda, SM. 1993. Role of integrins and other cell adhesion molecules in tumor progression and metastasis. *Lab. Invest.* 68:4–17

Albelda, SM, Mette, SA, Elder, DE, Stewart, RM, Damjanovich, L, et al. 1990. Integrin distribution in malignant melanoma: association of the β-3-subunit with tumor progession. *Cancer Res.* 50:6757–64

Albini, A, Melchiori, A, Santi, L, Liotta, LA, Brown, PD, Stetler-Stevenson, WG. 1991. Tumor cell invasion inhibited by TIMP-2. *J. Natl. Cancer Inst.* 83:775–79

Alvarez, OA, Carmichael, DF, DeClerck, YA. 1990. Inhibition of collagenolytic activity and metastasis of tumor cells by a recombinant human tissue inhibitor of metalloproteinases. *J. Natl. Cancer Inst.* 82:589–95

Aruffo, A, Stamenkovic, E, Melnick, M, Underhill, CB, Seed, B. 1990. CD44 is the principal cell surface receptor for hyaluronate. *Cell* 61:1303–13

Atnip, KD, Carter, LM, Nicolson, GL, Dabbous, MK. 1987. Chemotactic response of rat mammary adenocarcinoma cell clones to tumor-derived cytokines. *Biochem. Biophys. Res. Commun.* 146:996–1002

Aznavoorian, S, Stracke, ML, Krutzsch, HC, Schiffman, E, Liotta, LA. 1990. Signal transduction for chemotaxis and haptotaxis by matrix macromolecules in tumor cells. *J. Cell Biol.* 110:1427–38

Basset, P, Bellocq, JP, Wolf, C, Stoll, I, Hutin, P, et al. 1990. A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinoma. *Nature* 348:699–704

Behrens, J, Mareel, MM, Van Roy, FM, Birchmeier, W. 1989. Dissecting tumor cell invasion: Epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. *J. Cell. Biol.* 108: 2435–47

Birch, M, Mitchell, S, Hart, IR. 1991. Isolation and characterization of human melanoma cell variants expressing high and low levels of CD44. *Cancer Res.* 51:6660–67

Bishop, JM. 1991. Molecular themes in oncogenesis. *Cell* 64:235–48

Boag, AH, Young, ID. 1993. Immunohistochemical analysis of type IV collagenase expression in prostatic hyperplasia and adenocarcinoma. *Mod. Pathol.* 6:65–68

Bonfil, RD, Medina, PA, Gomez, DE, Farias, E, Lazarowski, A, et al. 1992. Expression of gelatinase/type IV collagenase in tumor necrosis correlates with cell detachment and tumor invasion. *Clin. Exp. Metastasis* 10: 211–20

Bonfil, RD, Reddel, RR, Ura, H, Reich, R, Fridman, R, et al. 1989. Invasive and metastatic potential of a v-Ha-*ras*-transformed human bronchial epithelial cell line. *J. Natl. Cancer Inst.* 81:587–94

Boone, TC, Johnson, MJ, DeClerck, YA, Langley, KE. 1990. cDNA cloning and expression of a metalloproteinase inhibitor related to tissue inhibitor of metalloproteinases. *Proc. Natl. Acad. Sci. USA* 87:2800–4

Brown, PD, Bloxidge, RE, Anderson, E, Howell, A. 1993a. Expression of activated gelatinase in human breast carcinoma. *Clin. Exp. Metastasis* 11:183–89

Brown, PD, Bloxidge, RE, Stuart, NSA, Gatter, KC, Carmichael, J. 1993b. Association between expression of activated 72-kilodalton gelatinase and tumor spread in non-small-cell lung carcinoma. *J. Natl. Cancer Inst.* 85:574–78

Brown, PD, Kleiner, DE, Unsworth, EJ, Stetler-Stevenson, WG. 1993c. Cellular activation of the 72 kDa type IV procollagenase/TIMP-2 complex. *Kidney Int.* 43:163–70

Brown, PD, Levy, AT, Margulies, IM, Liotta, LA, Stetler-Stevenson, WG. 1990. Independent expression and cellular processing of M_r 72,000 type IV collagenase and interstitial collagenase in human tumorigenic cell lines. *Cancer Res.* 50:6184–91

Campo, E, Merino, MJ, Liotta, L, Neumann, R, Stetler-Stevenson, WG. 1992a. Distribution of the 72-kd type IV collagenase in nonneoplastic and neoplastic thyroid tissue. *Hum. Pathol.* 23:1395–1401

Campo, E, Merino, MJ, Tavassoli, FA, Charonis, AS, Stetler-Stevenson, WG, Liotta, LA. 1992b. Evaluation of basement membrane components and the 72 kDa type IV collagenase in serous tumors of the ovary. *Am. J. Surg. Pathol.* 16:500–7

Carmichael, DF, Sommer, A, Thompson, RC, Anderson, DC, Smith, CG, et al. 1986. Primary structure and cDNA cloning of human fibroblast collagenase inhibitor. *Proc. Natl. Acad. Sci. USA* 83:2407–11

Cawston, TE, Galloway, WA, Mercer, E, Murphy, G, Reynolds, JJ. 1981. Purification of rabbit bone inhibitor of collagenase. *Biochem. J.* 195:159–65

Clavel, C, Polette, M, Doco, M, Binninger, I, Birembaut, P. 1992. Immunolocalization of matrix metallo-proteinases and their tissue inhibitor in human mammary pathology. *Bull. Cancer* 79:261–70

Collier, IE, Wilhelm, SM, Eisen, AZ, Marmer, BL, Grant, GA, et al. 1988. H-*ras* oncogene-transformed human bronchial ep-

ithelial cells (TBE-1) secrete a single metalloprotease capable of degrading basement membrane collagen. *J. Biol. Chem.* 263:6579–87

Coman, DR. 1953. Mechanisms responsible for the origin and distribution of bloodborne tumor metastases: a review. *Cancer Res.* 13:397–404

Condeelis, J, Jones, J, Segall, JE. 1992. Chemotaxis of metastatic tumor cells: Clues to mechanisms from the *Dictyostelium* paradigm. *Cancer Metastasis Rev.* 11:55–68

Cornil, I, Theodorescu, D, Man, S, Herlyn, M, Jambrosic, J, Kerbel, RS. 1991. Fibroblast cell interactions with human melanoma cells affect tumor cell growth as a function of tumor progression. *Proc. Natl. Acad. Sci. USA* 88:6028–32

Culty, M, Miyake, K, Kincade, PW, Silorski, E, Butcher, EC, Underhill, C. 1990. The hyaluronate receptor is a member of the CD44 (H-CAM) family of cell surface glycoproteins. *J. Cell. Biol.* 111:2765–74

Cunningham, CC. 1992. Actin structural proteins in cell motility. *Cancer Metastasis Rev.* 11:69–77

Daidone, MG, Silvestrini, R, D'Errico, A, Di Fr
receptors, collagenase IV and prognosis in node-negative breast cancers. *Int. J. Cancer* 48:529–32

Davies, B, Miles, DW, Happerfield, LC, Naylor, MS, Bobrow, LG, et al. 1993. Activity of type IV collagenases in benign and malignant breast disease. *Br. J. Cancer.* In press

DeClerck, YA, Perez, N, Shimada, H, Boone, TC, Langley, KE, Taylor, SM. 1992a. Inhibition of invasion and metastasis in cells transfected with an inhibitor of metalloproteinases. *Cancer Res.* 52:701–8

DeClerck, Y, Szpirer, C, Aly, MS, Cassiman, J-J, Eeckhout, Y, Rousseau, G. 1992b. The gene for tissue inhibitor of metalloproteinases-2 is localized on human chromosome arm 17q25. *Genomics* 14:782–84

DeClerck, YA, Yean, T-D, Chan, D, Shimada, H, Langley, KE. 1991. Inhibition of tumor invasion of smooth muscle cell layers by recombinant human metalloproteinase inhibitor. *Cancer Res.* 51:2151–57

DeClerck, YA, Yean, T-D, Ratzkin, BJ, Lu, HS, Langley, KE. 1989. Purification and characterization of two related but distinct metalloproteinase inhibitors secreted by bovine aortic endothelial cells. *J. Biol. Chem.* 264:17445–53

D'Errico, A, Garbisa, S, Liotta, LA, Castronovo, V, Stetler-Stevenson, WG, Grigioni, WF. 1991. Augmentation of type IV collagenase, laminin receptor, and Ki67 proliferation antigen associated with human colon, gastr
gression. *Mod. Pathol.* 4:239–46

Elices, MJ, Osborn, L, Takada, Y, Crouse, C, Luhowsky, S, et al. 1990. VCAM-1 on activated endothelium interacts with the leukocyte intefrin VLA-4 at a site distinct from the VLA-4 fibronectin binding-site. *Cell* 60:577–84

Emonard, HP, Remacle, AG, Nöel, AC, Grimaud, JA, Stetler-Stevenson, WG, Foidart, JM. 1992. Tumor cell surface-associated binding site for the M_r 72,000 type IV collagenase. *Cancer Res.* 52:5845–48

Enterline, HT, Coman, DR. 1950. The amoeboid motility of human and animal neoplastic cells. *Cancer* 3:1033–38

Fearon, ER, Vogelstein, B. 1990. A genetic model for colorectal tumorigenesis. *Cell* 61: 759–67

Fidler, IJ. 1991. Orthotopic implantation of human colon carcinomas into nude mice provides a valuable model for the biology and therapy of metastasis. *Cancer Metastasis Rev.* 10:229–43

Fidler, IJ, Har
in metastatic neoplasms-origins and implications. *Science* 217:998–1001

Fidler, IJ, Naito, S, Pathak, S. 1990. Orthotopic implantation is essential for the selection, growth and metastasis of human renal cell cancer in nude mice. *Cancer Metastasis Rev.* 9:149–65

Fidler, IJ, Radinsky, R. 1990. Genetic control of cancer metastasis. *J. Natl. Cancer Inst.* 82:166–68

Fridman, R, Fuerst, TR, Bird, RE, Hoyhtya, M, Oelkuct, M, et al. 1992a. Domain structure of human 72-kDa gelatinase/type IV collagenase. Characterization of proteolytic activity and identification of the tissue inhibitor of metalloproteinase-2 (TIMP-2) binding regions. *J. Biol. Chem.* 267:15398–15405

Fridman, R, Kibbey, MC, Royce, LS, Zain, M, Sweeney, TM, et al. 1991. Enhanced tumor growth of both primary and established human and murine tumor cells in athymic mice after coinjection with Matrigel. *J. Natl. Cancer Inst.* 83:769–74

Fridman, R, Sweeney, TM, Zain, M, Martin, GR, Kleinman, HK. 1992b. Malignant transformation of NIH-3T3 cells after subcutaneous co-injection with a reconstituted basement membrane (matrigel). *Int. J. Cancer* 51:740–44

Furukawa, T, Fu, X, Kubota, T, Watanabe, M, Kitajima, M, Hoffman, RM. 1993. Nude mouse metastatic models of human stomach cancer constructed using orthotopic implantation of histologically intact tissue. *Cancer Res.* 53:1204–8

Gallatin, WM, Rosenman, SJ, Ganji, A, St John, TP. 1991. Structure function relation-

ships of the CD44 class of glycoproteins. *Cell Mol. Mech. Inf.* 2:131–50

Garbisa, S, Pozzatti, R, Muschel, R, Saffioti, V, Ballin, M, et al. 1987. Secretion of type IV collagenolytic protease and metastatic phenotype: induction by transfection with c-Ha-*ras* but not c-Ha-*ras* plus Ad2-E1a. *Cancer Res.* 47:1523–28

Garbisa, S, Scagliotti, G, Masiero, L, Di Francesco, C, Caenazzo, C, et al. 1992. Correlation of serum metalloproteinase levels with lung cancer metastasis and response to therapy. *Cancer Res.* 52:4548–49

Garcia-Castro, I, Mato, JM, Vasanthokumar, G, Wiesmann, WP, Schiffmann, E, Chiang, PK. 1983. Paradoxical effects of adenosine on neutrophil chemotaxis. *J. Biol. Chem.* 258: 4345–49

Gehlsen, KR, Argraves, WS, Piersbacher, MD, Ruoslahti, E. 1988. Inhibition of in vitro cell invasion by Arg-Gly-Asp-containing peptides. *J. Cell Biol.* 106: 925–30

Gehlsen, KR, Davis, GE, Sriramarao, P. 1992. Integrin expression in human melanoma cells with differing invasive and metastatic properties. *Clin. Exp. Metastasis* 10: 111–20

Gladson, CL, Cherech, DA. 1991. Glioblastoma expression of vitronectin and the alpha-v/β-3 integrin. *J. Clin. Invest.* 88: 1924–32

Goldberg, GI, Marmer, BL, Grant, GA, Eisen, AZ, Wilhelm, A, He, C. 1989. Human 72-kilodalton type IV collagenase forms a complex with a tissue inhibitor of metalloproteinases designated TIMP-2. *Proc. Natl. Acad. Sci. USA* 86:8207–11

Goldberg, GI, Strongin, A, Collier, IE, Genrich, LT, Marmer, BL. 1992. Interaction of 92-kDa type IV collagenase with the tissue inhibitor of metalloproteinases prevents dimerization, complex formation with interstitial collagenase, and activation of the proenzyme with stromelysin. *J. Biol. Chem.* 267:4583–91

Goldman, DW, Chang, FH, Gifford, LA, Goetzl, EJ, Bourne, HR. 1985. Pertussis toxin inhibition of chemotactic factor-induced calcium mobilization and function in human polymorphonuclear leukocytes. *J. Exp. Med.* 162:145–56

Gray, ST, Wilkins, RJ, Yun, K. 1992. Interstitial collagenase gene expression in oral squamous cell carcinoma. *Am. J. Pathol.* 141:301–6

Guirguis, R, Margulies, I, Taraboletti, G, Schiffman, E, Liotta, LA. 1987. Cytokine-induced pseudopodial protrusion is coupled to tumor cell migration. *Nature* 329:261–63

He, C, Wilhelm, SM, Pentland, AP, Marmer, BL, Grant, GA, et al. 1989. Tissue cooperation in a proteolytic cascade activating human interstitial collagenase. *Proc. Natl. Acad. Sci. USA* 86:2632–36

Hewitt, RE, Leach, IH, Powe, DG, Clark, IM, Cawston, TE, Turner, DR. 1991. Distribution of collagenase and tissue inhibitor of metalloproteinases (TIMP) in colorectal tumours. *Int. J. Cancer* 49:666–72

Horst, E, Meijer, CJ, Radaszkiewicz, T, Osseloppele, GJ, Van Krieken, JH, Pals, ST. 1990. Adhesion molecules in the prognosis of diffuse large-cell lymphoma: expression of a lymphocyte homing receptor (CD44), LFA-1, (CD11a/18) and ICAM-1 (CD54). *Leukemia* 4:595–99

Hosaka, S, Suzuki, M, Sato, H. 1979. Leukocyte-like motility of cancer cells, with reference to the mechanism of extravasation. *Gann* 70:559–61

Howard, EW, Banda, MJ. 1991. Binding of tissue inhibitor of metalloproteinases 2 to two distinct sites on human 72-kDa gelatinase. Identification of a stabilization site. *J. Biol. Chem.* 266:17972–77

Howard, EW, Bullen, EC, Banda, MJ. 1991. Preferential inhibition of 72- and 92-kDa gelatinases by tissue inhibitor of metalloproteinases-2. *J. Biol. Chem.* 266:13070–75

Humphries, MJ, Olden, K, Yamada, KM. 1986. A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. *Science* 233:467–69

Humphries, MJ, Yamada, KM, Olden, K. 1988. Investigation of the biological effects of anti-cell adhesion synthetic peptides that inhibit experimental metastasis of B16-F10 murine melanoma cells. *J. Clin. Invest.* 81:782–90

Hunkapiller, TH, Hood, L. 1989. Diversity of the immunoglobulin superfamily. *Adv. Immunol.* 44:1–63

Hurewitz, AN, Zucker, S, Mancuso, P, Wu, CL, Dimassimo, B, et al. 1992. Human pleural effusions are rich in matrix metalloproteinases. *Chest* 102:1808–14

Hynes, RO. 1992. Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell* 69:11–25

Jouanneau, J, Gavrilovic, J, Caruelle, D, Jaye, M, Moens, G, et al. 1991. Secreted or nonsecreted forms of acidic fibroblast growth factor produced by transfected epithelial cells influence cell morphology, motility and invasive potential. *Proc. Natl. Acad. Sci. USA* 88:2893–97

Kahan, DW, Kramp, DC. 1987. Nerve growth factor stimulation of mouse embryonal cell migration. *Cancer Res.* 47:6324–28

Kanemoto, T, Martin, GR, Hamilton, TC, Fridman, R. 1991. Effects of synthetic peptides and protease inhibitors on the interaction of a human ovarian cancer cell line (NIH:OVCAR-3) with a reconstituted base-

ment membrane (Matrigel). *Invasion Metastasis* 11:84–92

Karaoka, H, DeCastro, R, Zucker, S, Biswas, C. 1993. The tumor cell-derived collagenase stimulatory factor, TCSF, increases expression of interstitial collagenase, stromelysin and 72 kDa gelatinase. *Cancer Res.* In press

Kato, Y, Nakayama, Y, Umeda, M, Miyazaki, K. 1992. Induction of 103-kDa gelatinase/type IV collagenase by acidic culture conditions in mouse metastatic melanoma cell lines. *J. Biol. Chem.* 267: 11424–30

Kerbel, R. 1990. Growth dominance of the metastatic cancer cell: cellular and molecular aspects. *Adv. Cancer Res.* 55:87–131

Kerbel, R. 1992. Expression of multicytokine resistance and multi-growth factor independence in advanced stage metastatic cancer. *Am. J. Pathol.* 141:519–24

Khokha, R, Waterhouse, P, Yagel, S, Lala, PK, Overall, CM, et al. 1989. Antisense RNA-induced reduction in metalloproteinase inhibitor causes mouse 3T3 cells to become tumorigenic. *Science* 243:947–50

Kleiner, DE Jr, Tuuttila, A, Tryggvason, K, Stetler-Stevenson, WG. 1993. Stability analysis of latent and active 72-kDa type IV collagenase: the role of tissue inhibitor of metalloproteinases-2 (TIMP-2). *Biochemistry* 32:1583–92

Kleiner, DE Jr, Unsworth, EJ, Krutzsch, HC, Stetler-Stevenson, WG. 1992. Higher-order complex formation between the 72-kilodalton type IV collagenase and tissue inhibitor of metalloproteinases-2. *Biochemistry* 31:1665–72

Kusukawa, J, Sasaguri, Y, Shima, I, Kameyama, T, Morimatsu, M. 1993. Expression of matrix metalloproteinase-2 related to lymph node metastasis of oral squamous cell carcinoma. A clinicopathologic study. *Am. J. Clin. Pathol.* 99:18–23

Lefebvre, O, Wolf, C, Limacher, J-M, Hutin, P, Wendling, C, et al. 1992. The breast cancer-associated stromelysin-3 gene is expressed during mouse mammary gland apoptosis. *J. Cell Biol.* 119:997–1002

Lester, BR, McCarthy, JB. 1992. Tumor cell adhesion to the extracellular matrix and signal transduction mechanisms implicated in tumor cell motility, invasion and metastasis. *Cancer Metastasis Rev.* 11:31–44

Lester, BR, McCarthy, JB, Sun, Z, Smith, RS, Furcht, KT, Spiegel, AM. 1989. G-protein involvement in matrix-mediated motility and invasion of high and low experimental metastatic B16 melanoma clones. *Cancer Res.* 49:5940–48

Lester, BR, Winstein, LS, McCarthy, JB, Sun, Z, Smith, RS, Furcht, LT. 1991. The role of G-protein in matrix-mediated motlilty of highly and poorly invasive melanoma cells. *Int. J. Cancer* 48:113–20

Levy, AT, Cioce, V, Sobel, ME, Garbisa, S, Grigioni, WF, et al. 1991. Increased expression of the M_r 72,000 type IV collagenase in human colonic adenocarcinoma. *Cancer Res.* 51:439–44

Liotta, LA. 1986. Tumor invasion and metastases-role of the extracellular matrix: Rhoads Memorial Award Lecture. *Cancer Res.* 46:1–7

Liotta, LA, Mandler, R, Murano, G, Katz, DA, Gordon, RK, et al. 1986. Tumor cell autocrine motility factor. *Proc. Natl. Acad. Sci. USA* 83:3302-6

Liotta, LA, Steeg, PS, Stetler-Stevenson, WG. 1991. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* 64:327–36

Lyons, JG, Birkedal-Hansen, B, Moore, WG, O'Grady, RL, Birkedal-Hansen, H. 1991. Characteristics of a 95-kDa matrix metalloproteinase produced by mammary carcinoma cells. *Biochemistry* 30:1449–56

Mahtani, MM, Willard, HF. 1988. A primary genetic map of the pericentormeric region of the human X chromosome. *Genomics* 2:294–301

Manzotti, C, Audisio, RA, Pratesi, G. 1993. Importance of orthotopic implantation for human tumors as model systems: relevance to metastasis and invasion. *Clin. Exp. Metastasis* 11:5–14

Marcotte, PA, Kozan, IM, Dorwin, SA, Ryan, JM. 1992. The matrix metalloproteinase pump-1 catalyzes formation of low molecular weight (Pro)urokinase in cultures of normal human kidney cells. *J. Biol. Chem.* 267:13803–6

Mareel, MM, Van Roy, FM, De Baetselier, P. 1990. The invasive phenotypes. *Cancer Metastasis Rev.* 9:45–62

Margulies, IMK, Hoyhtya, M, Evans, C, Stracke, ML, Liotta, LA, Stetler-Stevenson, WG. 1992. Urinary type IV collagenase: elevated levels are associated with bladder transitional cell carcinoma. *Cancer Epidemiol. Biomarkers Prev.* 1:467–74

Martignone, S, Menard, S, Bufalino, R, Cascinelli, N, Pellegrini, R, et al. 1993. Prognostic significance of the 67-kilodalton laminin receptor expression in human breast carcinomas. *J. Natl. Cancer Inst.* 85:398–402

Matrisian, LM. 1992. The matrix-degrading metalloproteinases. *BioEssays* 14:455–62

Matrisian, LM, McDonnell, S, Miller, DB, Navre, M, Seftor, EA, Hendrix, MJ. 1991. The role of the matrix metalloproteinase stromelysin in the progression of squamous

cell carcinomas. *Am. J. Med. Sci.* 302:157–62

McCarthy, JB, Basara, ML, Palm, SL, Sas, DF, Furcht, LT. 1985. The role of cell adhesion proteins-laminin and fibronectin-in the movement of malignant and metastatic cells. *Cancer Metastasis Rev.* 4:125–52

McCarthy, JB, Furcht, LT. 1984. Laminin and fibronectin promote the haptotactic migration of B16 melanoma cells in vitro. *J. Cell Biol.* 98:1474–80

McCarthy, JB, Hager, SJ, Burcht, LR. 1986. Human fibronectin contains distinct adhesion- and motility-promoting domains for metastatic melanoma cells. *J. Cell Biol.* 102:179–88

McDonnell, S, Navre, M, Coffey, R Jr, Matrisian, LM. 1991. Expression and localization of the matrix metalloproteinase pump-1 (MMP-7) in human gastric and colon carcinomas. *Mol. Carcinog.* 4:527–33

Meltzer, A. 1990. Dormancy and breast cancer. *J. Surg. Oncol.* 43:181–88

Mignatti, P, Robbins, E, Rifkin, DB. 1986. Tumor invasion through the human amnion membrane: requirement for a proteinase cascade. *Cell* 47:487–98

Mohler, JL, Partin, AW, Coffey, DS. 1987. Prediction of metastatic potential by a new grading system of cell motility: validation in the Dunning R-3327 prostatic adenomcarcinoma model. *J. Urol.* 138:168–70

Monteagudo, C, Merino, MJ, San Juan, J, Liotta, LA, Stetler-Stevenson, WG. 1990. Immunohistochemical distribution of type IV collagenase in normal, benign, and malignant breast tissue. *Am. J. Pathol.* 136: 585–92

Montesano, R, Pepper, MS, Mohle-Steinlein, U, Risau, W, Wagner, WF, Orci, L. 1990. Increased proteolytic activity is responsible for the aberrant morphogenetic behavior of endothelial cells expressing the middle T oncogene. *Cell* 62:435–45

Moses, MA, Langer, R. 1991. A metalloproteinase inhibitor as an inhibitor of neovascularization. *J. Cell. Biochem.* 47: 230–35

Moses, MA, Sudhalter, J, Langer, R. 1990. Identification of an inhibitor of neovascularization from cartilage. *Science* 248:1408–10

Muller, D, Breathnach, R, Engelmann, A, Millon, R, Bronner, G, et al. 1991. Expression of collagenase-related metalloproteinase genes in human lung or head and neck tumours. *Int. J. Cancer* 48:550–56

Muller, D, Wolf, C, Abecassis, J, Millon, R, Engelmann, A, et al. 1993. Increased stromelysin 3 gene expression is associated with increased local invasiveness in head and neck squamous cell carcinomas. *Cancer Res.* 53:165–69

Murphy, G, Ward, R, Gavrilovic, J, Atkinson, S. 1992a. Physiological mechanisms for metalloproteinase activation. *Matrix* 1: 224–30

Murphy, G, Willenbrock, F, Ward, RV, Cockett, MI, Eaton, D, Docherty, AJ. 1992b. The C-terminal domain of 72 kDa gelatinase A is not required for catalysis, but is essential for membrane activation and modulates interactions with tissue inhibitors of metalloproteinases. *Biochem. J.* 283: 637–41

Muschel, RJ, Williams, JE, Lowy, DR, Liotta, LA. 1985. Harvey *ras* induction of metastatic potential depends upon oncogene activation and the type of recipient cell. *Am. J. Pathol.* 121:1–8

Nabeshima, K, Lane, WS, Biswas, C. 1991. Partial sequencing and characterization of the tumor cell-derived collagenase stimulatory factor. *Arch. Biochem. Biophys.* 285: 90–96

Nagase, H, Ogata, Y, Suzuki, K, Enghild, JJ, Salvesen, G. 1991. Substrate specificities and activation mechanisms of matrix metalloproteinases. *Biochem. Soc. Trans.* 19:715–18

Nakajima, M, Morikawa, K, Fabra, A, Bucana, CD, Fidler, IJ. 1990. Influence of organ environment on extracellular matrix degradative activity and metastasis of human colon carcinoma cells. *J. Natl. Cancer Inst.* 82:1890–98

Nakajima, M, Welch, DR, Belloni, PN, Nicholson, GL. 1987. Degradation of basement membrane type IV collagen and lung subendothelial matrix by rat mammary adenocarcinoma cell clones of differing metastatic potentials. *Cancer Res.* 47:4869–76

Ogata, Y, Enghild, JJ, Nagase, H. 1992a. Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. *J. Biol. Chem.* 267: 3581–84

Ogata, Y, Pratta, MA, Nagase, H, Arner, EC. 1992b. Matrix metalloproteinase 9 (92-kDa gelatinase/type IV collagenase) is induced in rabbit articular chondrocytes by cotreatment with interleukin 1 beta and a protein kinase C activator. *Exp. Cell Res.* 201:245–49

Ohnishi, R, Arita, N, Hayakawa, T, Izumoto, S, Taki, T, Yamamoto, H. 1990. Motility factor produced by malignant glioma cells: role in tumor invasion. *J. Neurosurg.* 73: 881–88

Ohori, NP, Yousem, SA, Griffin, J, Stanis, K, Stetler-Stevenson, WG, et al. 1992. Comparison of extracellular matrix antigens in subtypes of bronchioloalveolar carcinoma and conventional pulmonary adenocarci-

noma. An immunohistochemical study. *Am. J. Surg. Pathol.* 16:675–86

Okada, Y, Morodomi, T, Enghild, JJ, Suzuki, K, Yasui, A, et al. 1990. Matrix metalloproteinase 2 from human rheumatoid synovial fibroblasts. *Eur. J. Biochem.* 194: 721–30

Osborn, L, Hession, C, Tizard, R, Vassallo, C, Luhowsky, S, et al. 1989. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell* 59: 1203–11

Otani, Y. 1990. The collagenase activities, interstitial collagenase and type IV collagenase, in human stomach cancer: with special reference to local spreading and lymph node metastasis. *Keio J. Med.* 39: 159–67

Overall, CM, Sodek, J. 1990. Concanavalin A produces a matrix-degradative phenotype in human fibroblasts. Induction and endogenous activation of collagenase, 72-kDa gelatinase, and Pump-1 is accompanied by the suppression of the tissue inhibitor of matrix metalloproteinases. *J. Biol. Chem.* 265:21141–51

Pajouh, MS, Nagle, RB, Breathnach, R, Finch, JS, Brawer, MK, Bowden, GT. 1991. Expression of metalloproteinase genes in human prostate cancer. *J. Cancer Res. Clin. Oncol.* 117:144–50

Pauli, BU, Augustin-Voss, HG, El-Sabban, ME, Johnson, RC, Hammer, DA. 1990. Organ-preference of metastasis. The role of endothelial cell adhesion molecules. *Cancer Metastasis Rev.* 9:175–89

Pepper, MS, Belin, D, Montesano, R, Orci, L, Vassalli, J-D. 1990. Transforming growth factor-beta 1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells in vitro. *J. Cell Biol.* 111:743–55

Petersen, OE, Ronnov-Jessen, L, Howlett, AR, Bissell, MJ. 1992. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. *Proc. Natl. Acad. Sci. USA* 89:9064–68

Polette, M, Clavel, C, Muller, D, Abecassis, J, Binninger, I, Birembaut, P. 1991. Detection of mRNAs encoding collagenase I and stromelysin 2 in carcinomas of the head and neck by in situ hybridization. *Invasion Metastasis* 11:76–83

Poulsom, R, Pignatelli, M, Stetler-Stevenson, WG, Liotta, LA, Wright, PA, et al. 1992. Stromal expression of 72 kda type IV collagenase (MMP-2) and TIMP-2 mRNAs in colorectal neoplasia. *Am. J. Pathol.* 141: 389–96

Powell, WC, Knox, JD, Navre, M, Grogan, TM, Kittelson, J, et al. 1993. Expression of the metalloproteinase matrilysin in DU-145 cells increases their invasive potential in severe combined immunodeficient mice. *Cancer Res.* 53:417–22

Pyke, C, Ralfkiaer, E, Huhtala, P, Hurskainen, T, Danö, K, Tryggvason, K. 1992. Localization of messenger RNA for M_r 72,000 and 92,000 type IV collagenases in human skin cancers by in situ hybridization. *Cancer Res.* 52:1336–41

Rao, CN, Marguiles, IMK, Tralka, TS, Terranova, VP, Madri, JA, Liotta, LA. 1982. Isolation of a subunit of laminin and its role in molecular structure of tumor cell attachment. *J. Biol. Chem.* 257:9740–44

Rice, GE, Bevilavqua, MP. 1989. An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. *Science* 246: 1303–6

Rodeck, U, Herlyn, M, Menssen, HD, Furlanetto, RW, Koprowski, H. 1987. Metastatic but not primary melanoma cell lines grow in vitro independently of exogenous growth factors. *Int. J. Cancer* 40:687–90

Roos, E, Van De Pavert, IB. 1987. Inhibition of lymphoma invasion and liver metastasis formation by pertussis toxin. *Cancer Res.* 47:5439–44

Rosen, RM, Meromsky, L, Setter, E, Vinter, DW, Goldberg, ID. 1990. Purified scatter factor stimulates epithelial and vascular endothelial cell migration. *Proc. Soc. Exp. Biol. Med.* 195:34–43

Ruoslahti, E, Pierschbacher, MD. 1987. New perspectives in cell adhesion: RGD and integrins. *Science* 238:491–97

Sage, EH, Bornstein, P. 1991. Extracellular proteins that modulate cell-matrix interactions. *J. Biol. Chem.* 266:14831–34

Saiki, I, Murata, J, Iida, J, Nishi, J, Sugimura, K, Azuma, I. 1989. The inhibition of murine lung metastasis by synthetic polypeptides [poly(arg-gly-asp) and poly(tyr-ile-gly-ser-arg)] with a core sequence of cell adhesion molecules. *Br. J. Cancer* 59:194–97

Sato, H, Kida, Y, Mai, M, Endo, Y, Sasaki, T, et al. 1992. Expression of genes encoding type IV collagen-degrading metalloproteinases and tissue inhibitors of metalloproteinases in various human tumor cells. *Oncogene* 7:77–83

Schnapper, HW, Grant, DS, Stetler-Stevenson, WG, Fridman, R, D'Orazi, GD, et al. 1993. Type IV collagenase(s) and TIMPs modulate endothelial cell morphogenesis in vitro. *J. Cell. Physiol.* In press

Schor, SL, Schor, AM, Grey, AM, Rushton, G. 1988. Fetal and cancer patient fibroblasts produce an autocrine migration-stimulating factor not made by normal adult cells. *J. Cell Sci.* 90:391–99

Schultz, RM, Silberman, S, Persky, B, Bajkowski, AS, Carmichael, DF. 1988. Inhibition by human recombinant tissue inhibitor of metalloproteinases of human amnion invasion and lung colonization by murine B16-F10 melanoma cells. *Cancer Res.* 48: 5539–45

Seftor, RE, Seftor, EA, Gehlsen, KR, Stetler-Stevenson, WG, Brown, PD, et al. 1992. Role of the alpha-v-beta-3 integrin in human melanoma cell invasion. *Proc. Natl. Acad. Sci. USA* 89:1557–61

Seiki, M, Sato, H, Liotta, LA, Schiffmann, E. 1991. Comparison of autocrine mechanisms promoting motility in two metastatic cell lines: human melanoma and *ras*-transfected NIH3T3 cells. *Int. J. Cancer* 49:717–20

Shima, I, Sasaguri, Y, Kusukawa, J, Yamana, H, Fujita, H, et al. 1992. Production of matrix metalloproteinase-2 and metalloproteinase-3 related to malignant behavior of esophageal carcinoma. A clinicopathologic study. *Cancer* 70:2747–53

Siletti, S, Watanabe, H, Hogan, B, Nabi, IR, Raz, A. 1992. Purification of B16-F10 melanoma autocrine motility factor and its receptor. *Cancer Res.* 51:3507-11

Spangrude, GJ, Sacchi, G, Hill, JR, Van Epps, DE, Daynes, RA. 1985. Inhibition of lymphocyte and neutrophil chemotaxis by pertussis toxin. *Cancer Res.* 135:4135–56

Sreenath, T, Matrisian, LM, Stetler-Stevenson, W, Gattoni-Celli, S, Pozzatti, RO. 1992. Expression of matrix metalloproteinase genes in transformed rat cell lines of high and low metastatic potential. *Cancer Res.* 52:4942–47

Stamenkovic, I, Amiot, M, Pesando, JM, Seed, B. 1989. A lymphocyte molecule implicated in lymph node homing is a member of the cartilage-like protein family. *Cell* 56:1057–62

Stephenson, RA, Dinney, CP, Gohji, K, Ordonez, NG, Killion, JJ, Fidler, IJ. 1992. Metastatic model for human prostate cancer using orthotopic implantation in nude mice. *J. Natl. Cancer Inst.* 84:951–57

Stetler-Stevenson, WG, Brown, PD, Onisto, M, Levy, AT, Liotta, LA. 1990. Tissue inhibitor of metalloproteinases-2 (TIMP-2) mRNA expression in tumor cell lines and human tumor tissues. *J. Biol. Chem.* 265:13933–38

Stetler-Stevenson, WG, Krutzsch, HC, Liotta, LA. 1989. Tissue inhibitor of metalloproteinase (TIMP-2). *J. Biol. Chem.* 264: 17374–78

Stetler-Stevenson, WG, Liotta, LA, Seldin, MF. 1992. Linkage analysis demonstrates that the Timp-2 locus is on mouse chromosome 11. *Genomics* 14:828–29

Stossel, TP. 1990. How cells crawl, with the discovery that the cellular motor contains muscle protein, we can begin to describe cell motility in molecular detail. *Am. Sci.* 78:408–23

Stracke, ML, Engel, JD, Wilson, LL, Rechler, MM, Liotta, LA, Schiffman, E. 1989. The type I insulin-like growth factor receptor is a motility receptor in human melanoma cells. *J. Biol. Chem.* 264:1544–49

Stracke, ML, Guirguis, R, Liotta, LA, Schiffman, E. 1987. Pertussis toxin inhibits stimulated motility independently of the adenylate cyclase pathway in human melanoma cells. *Biochem. Biophys.Res. Commun.* 146:339–45

Stracke, ML, Kohn, EC, Aznavoorian, S, Wilson, LL, Salomon, D, et al. 1988. Insulin-like growth factors stimulate chemotaxis in human melanoma cells. *Biochem. Biophys. Res. Commun.* 153:1076–83

Stracke, ML, Krutzsch, HC, Unsworth, EJ, Arestad, A, Cioce, V, et al. 1992. Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein. *J. Biol. Chem.* 267:2524–29

Sweeney, TM, Kibbey, MC, Zain, M, Fridman, R, Kleinman, HK. 1991. Basement membrane and the SIKVAV laminin-derived peptide promote tumor growth and metastases. *Cancer Metastasis Rev.* 10:245–54

Takeichi, M. 1990. Cadherins: A molecular family important in selective cell-cell adhesion. *Annu. Rev. Biochem.* 59:237–52

Takigawa, M, Nishida, Y, Suzuki, F, Kishi, J, Yamashita, K, Hayakawa, T. 1990. Induction of angiogenesis in chick yolk-sac membrane by polyamines and its inhibition by tissue inhibitors of metalloproteinases (TIMP and TIMP-2). *Biochem. Biophys. Res. Commun.* 171:1264–71

Taraboletti, G, Roberts, DD, Liotta, LA. 1987. Thrombospondin-induced tumor cell migration: haptotaxis and chemotaxis are mediated by different domains. *J. Cell Biol.* 105:2409–15

Templeton, NS, Brown, PD, Levy, AT, Margulies, IM, Liotta, LA, Stetler-Stevenson, WG. 1990. Cloning and characterization of human tumor cell interstitial collagenase. *Cancer Res.* 50:5431–37

Testa, J. 1992. Loss of the metastatic phenotype by a human epidermoid carcinoma cell line HEp-3, is accompanied by increased expression of tissue inhibitor of metalloproteinase-2. *Cancer Res.* 52:5597–5603

Tsuboi, R, Rifkin, DB. 1990. Bimodal relationship between invasion of the amniotic membrane and plasminogen activator activity. *Int. J. Cancer* 46:56–60

Turley, EA. 1992. Hyaluronan and cell locomotion. *Cancer Metastasis Rev.* 11:21–30

Turley, EA, Austen, L, Vandeligt, K, Clary, C. 1991. Hyaluronan and a cell-associated hyaluronan binding protein regulate the locomotion of *ras*-transformed cells. *J. Cell. Biol.* 112:1041–1047

Uleminckx, K, Vackat, L, Mareel, M, Fiers, W, Van Roy, FV. 1991. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell* 66:107–19

Urbanski, SJ, Edwards, DR, Maitland, A, Leco, KJ, Watson, A, Kossakowska, AE. 1992. Expression of metalloproteinases and their inhibitors in primary pulmonary carcinomas. *Br. J. Cancer* 66:1188–94

van der Stappen, JWJ, Hendriks, T, Wobbes, T. 1990. Correlation between collagenolytic activity and grade of histological differentiation in colorectal tumors. *Int. J. Cancer* 45:1071–1078

Ward, RV, Atkinson, SJ, Slocombe, PM, Docherty, AJP, Reynolds, JJ, Murphy, G. 1991. Tissue inhibitor of metalloproteinases-2 inhibits the activation of 72 kDa progelatinase by fibroblast membranes. *Biochim. Biophys. Acta* 1079:242–46

Weidner, KM, Behrens, J, Vandekerckhove, J, Birchmeier, W. 1990. Scatter factor: molecular characteristics and effect on the invasiveness of epithelial cells. *J. Cell Biol.* 111:2097–2108

Weinberg, WC, Brown, PD, Stetler-Stevenson, WG, Yuspa, SH. 1990. Growth factors specifically alter hair follicle cell proliferation and collagenolytic activity alone or in combination. *Differentiation* 45:168–78

Welgus, HG, Stricklin, GP. 1983. Human skin fibroblast collagenase inhibitor. Comparative studies in human connective tissues, serum, and amniotic fluid. *J. Biol. Chem.* 258:12259–64

Wewer, UM, Liotta, LA, Jaye, M, Ricca, GA, Drohan, WN, et al. 1986. Altered levels of laminin receptor mRNA in various human carcinoma cells that have different abilities to bind laminin. *Proc. Natl. Acad. Sci. USA* 83:7137–41

Wewer, UM, Taraboletti, G, Sobel, ME, Albrechtsen, R, Liotta, LA. 1987. Role of laminin receptor in tumor cell migration. *Cancer Res.* 47:5691–98

Wilhelm, SM, Collier, IE, Marmer, BL, Eisen, AZ, Grant, GA, Goldberg, GI. 1989. SV40-transformed human lung fibroblasts secrete a 92-kDa type IV collagenase which is identical to that secreted by normal human macrophages. *J. Biol. Chem.* 264: 17213–21

Willard, HF, Durfy, SJ, Mahtani, MM, Dorkins, H, Davies, KE, Williams, BRG. 1989. Regional localization of the TIMP gene on the human X chromosome. *Hum. Genet.* 81:234–38

Williams, AF, Barclay, AN. 1988. The immunoglobulin superfamily—domains for cell surface recognition. *Annu. Rev. Immunol.* 6:381–405

Woessner, JF Jr. 1991. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J.* 5: 2145–54

Wolf, C, Chenard, M-P, de Grossouvre, PD, Bellocq, J-P, Chambon, P, Basset, P. 1992. Breast-cancer-associated stromelysin-3 gene is expressed in basal cell carcinoma and during cutaneous wound healing. *J. Invest. Dermatol.* 99:870–72

Yamada, KM. 1991. Adhesive recognition sequences. *J. Biol. Chem.* 266:12809–12

Yusa, R, Blood, CM, Zetter, BR. 1989. Tumor cell interactions with elastin: implication for pulmonary metastasis. *Am. Rev. Respir. Dis.* 140:1458–62

Zajicek, G. 1987. Long survival with micrometastases. At least 9% of breast cancer patients carry metastases for more than 10 years. *The Cancer J.* 1:414–15

Zhu, D, Cheng, C-F, Pauli, BU. 1991. Mediation of lung metastasis of murine melanomas by a lung-specific endothelial adhesion molecule. *Proc. Natl. Acad. Sci. USA* 88:9568–72

Zucker, S, Lysik, RM, Zarrabi, MH, Moll, U. 1993. M_r 92,000 type IV collagenase is increased in plasma of patients with colon cancer and breast cancer. *Cancer Res.* 53:140–46

Zucker, S, Lysik, RM, Zarrabi, MH, Stetler-Stevenson, WG, Liotta, LA, et al. 1992. Type IV collagenase/gelatinase (MMP-2) is not increased in plasma of patients with cancer. *Cancer Epidemiol. Biomarkers Prev.* 1:475–79