Grand Rounds at the Clinical Center of the National Institutes of Health

Saul Rosen, PhD, MD, *Section Editor*

Cancer Invasion and Metastases

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SELECTED CASE

EIGHT years ago, a 62-year-old woman sought evaluation for an asymptomatic 2-cm mammographic abnormality in the upper part of the outer quadrant of her left breast. The results of an incisional biopsy were indicative of malignancy. After staging evaluation revealed no distant metastatic disease, she underwent a modified radical mastectomy with axillary node dissection. At the time of pathological review, a 2×2-cm estrogen receptor–positive infiltrating ductal adenocarcinoma was found; axillary lymph nodes were free of tumor (stage II, T2, N0, M0).

Follow-up was uneventful until presentation 1 year ago, when the patient developed progressive dyspnea on exertion, orthopnea, a dry cough, and a heaviness in the left side of her chest. At the time of examination, she was tachypneic and tachycardiac, and physical findings included dullness to percussion and absent left-sided breath sounds; she was admitted for treatment. A chest roentgenogram showed an opacified left hemithorax and multiple nodular densities in the right lung fields, and hypoxemia was present in an arterial blood sample. Thoracentesis was performed, and bloody exudate (1.5 L) was removed for analysis. Cytological examination confirmed the diagnosis of recurrent breast cancer. After thoracostomy tube drainage, followed by successful pleurodesis, numerous nodules were seen in the underlying left lung. She was discharged and received tamoxifen citrate (10 mg twice a day).

After a 5-month response to tamoxifen with regression of many of the nodules, the patient developed progressive disease in the liver, bones, and brain. Despite other hormonal interventions and cytotoxic chemotherapy during the ensuing 7 months, the disease had a progressive course and the patient died 8 years after the initial diagnosis.

The most insidious aspect of cancer is its propensity to invade normal host tissue and to metastasize to distant organs. This is a major cause of cancer treatment failure. There have been a number of exciting discoveries that relate to the biochemical and genetic mechanisms of tumor progression to the metastatic phenotype. We provide an overview of some of these recent developments and their clinical implications.

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DISCUSSION

A metastatic colony is the end result of a complicated series of steps (Fig 1),[1] including separation of individual or groups of tumor cells from the primary tumor, entry into lymphatic and blood vessels, circulation of the tumor cells singly and in clumps, and arrest of the tumor cell[2] in the small vessels of the target organ. The arrested tumor cell, which may adhere to the surface of the luminal endothelium, may cause retraction of the endothelial cells, exposing the underlying subendothelial basement membrane, to which the tumor cell then attaches avidly. After invading through the basement membrane, the tumor cells exit from the circulation and can initiate a metastatic colony. For the colony to grow, extravasated tumor cells may use autocrine growth mechanisms or growth factors from the local tissue and the circulation.

For a metastatic colony to be initiated, a single tumor cell must leave the primary tumor site, traverse the previously mentioned barriers, overcome host defenses, and replicate at the secondary site. To do so, the successful tumor cell must possess the right combination of gene products, giving it the ability to accomplish all of these steps. Thus, the major challenge to investigators who study cancer metastases is to identify such necessary gene products that might serve as markers to help identify metastatic cells, to predict the aggressiveness of disease, and to locate and eradicate metastases.

Most patients who have newly identified cancer may already have metastatic disease. For many solid tumors, up to 60% of patients may have microscopic and/or clinically evident metastases at the time of primary tumor diagnosis. As in the index case of stage II node–negative breast cancer, approximately 20% to 30% of these patients have clinically occult or dormant metastases.[2]

Some primary tumors in humans have a characteristic organ distribution for their metastases. The organ distribution of metastases depends largely on the site of the primary tumor.[3] Fifty percent to 60% of the distribution of metastases can be predicted from the anatomic route followed by the disseminated tumor cell. Most occur in the first capillary or nodal bed that is encountered after tumor cells exit the primary tumor. For example, sarcomas tend to metastasize to the lungs because tumor cells from the venous drainage of the sarcoma are carried to the right side of the heart, enter the pulmonary circulation, and arrest in the lung. As cells from tumors that arise in the lung are carried to the left side of the heart and out to systemic organs, there is a high propensity of brain metastases that arise from primary tumors in the lung. Most tumors can metastasize by both lymphatic and vascular routes, and because of lymphatic hematogenous communications, lymphatic and hematogenous dissemination may occur in parallel. Thus, the presence of lymph node metastases may be a hallmark of general dissemination.

In approximately 40% of tumors, the distribution of metastases to various organs may not be predicted by anatomic considerations alone. This includes the ability of breast cancer to metastasize to the ovaries or for renal cell carcinoma to metastasize to the adrenal glands. For these diseases, a homing mechanism is likely to cause the tumor cells to metastasize preferentially to specific organs.[4] Three organ-homing mechanisms are thought to exist. First, tumor cells disseminate equally to all organs but preferentially grow only in those organs with the correct local growth factors. Second, tumor cells may adhere preferentially to the endothelial surface of the target organ. Following adherence to organ-specific determinants, the tumor cells extravasate to initiate a metastatic colony. The third mechanism for homing may involve soluble chemical signals that emanate from the secondary target tissue may attract the tumor cell to exit

the circulation and proceed along a positive chemical gradient (chemotaxis). Although factors that mediate specific homing mechanisms have not been isolated, all three of these mechanisms may play a role in different tumors.

In addition to the impact of site of metastases, another important problem is the aggressiveness of the individual tumor. Some tumors grow to a large size and never metastasize, whereas other tumors apparently metastasize at a very early stage in their growth. Consequently, major clinical objectives are accurate prediction of the metastatic propensity of a patient's tumor, localization of clinically silent micrometastases, and selective eradication of established metastases during treatment of the primary tumor. It is hoped that identification of biochemical or genetic alterations will provide markers that can be applied to these clinical problems.

How do investigators go about identifying biochemical factors involved in metastases, a complicated multistep process? Biochemical mechanisms have been investigated based on the strategy of breaking down the process of metastasis into steps and focusing on one step at a time. Significant progress has been made in understanding how tumor cells invade the extracellular matrix. The basement membrane is a continuous extracellular matrix that separates one tissue compartment from the next and surrounds blood vessels, nerves, muscle, endothelium, epithelium, and mesothelium. For the tumor cell to metastasize, it must penetrate the basement membrane of the host organ and that which surrounds the vascular endothelium at the site of the primary tumor and again at the secondary site. Based on ultrastructural studies, this invasion occurs in the following three steps[6]: first, adherence of the tumor cell to the basement membrane; second, local proteolysis associated with breakdown of the basement membrane components; and last, migration and locomotion of the tumor cell through the defect in the extracellular matrix.

Our laboratory and others have identified unique proteins that might play a role in each of these three steps. The laminin receptor, a cell-surface protein that binds the laminin component of the basement membrane, might play a role in the first adhesion step.[6] Type IV collagenase, a metalloprotease that specifically degrades the type IV collagen of the basement membrane, may be important for the second step.[7] A new class of cytokines, autocrine motility factors, may play a role in stimulating lomotion through the permeabilized basement membrane in the third step (Fig 1).[8]

The laminin receptor is a cell-surface protein that binds to laminin, a cross-shaped glycoprotein found only in basement membranes. Laminin, in turn, through its globular end regions, binds to the type IV collagen backbone of the basement membrane.[9] Thus, laminin serves as a bridge between the basement membrane and the tumor cell. Normal cells have laminin receptors that have been demonstrated to be occupied and polarized at the base of the cell while the cell is in contact with the basement membrane. In contrast, highly aggressive, actively invading tumor cells often have a content of laminin receptor that is increased, in some cases 50-fold, and is distributed uniformly over the cell surface.[10] Many laboratories have produced fragments of the laminin molecule that block the binding of laminin to its receptor. These fragments can be used to study whether the laminin receptor plays an important role in metastasis. In experiments that use murine tumors or human breast carcinoma cells, tumor cells injected into the tail vein of athymic mice form numerous pulmonary metastases. In contrast, when the tumor cells are pretreated with the laminin fragment, they fail to form metastatic colonies.[11,12] The gene for the laminin receptor has been cloned.[13] Using the laminin receptor clone and its predicted encoded protein, peptide-specific antibodies have been developed, which may serve as targeting agents to bring toxic factors directly to tumor cells that express high levels of laminin receptors. The laminin receptor may play an important role in the exit of the tumor cell from the circulation.

The second step in invasion is local degradation of the basement membrane, which may be facilitated by the production of proteases by the tumor cell. An important enzyme involved in degradation of the basement membrane is type IV collagenase.[7] Type IV collagenase shares homology with other metalloproteases, such as collagenase I and stromelysin.[14-16] However, the amino terminal side of the metal-binding domain of type IV collagenase shows no homology to the other metalloproteases[14] and may serve as the binding domain on the enzyme that selectively recognizes the type IV collagen substrate. Based on sequence information, peptide-specific antibodies that are targeted to defined domains of type IV collagenase have been generated.[16] Immunohistological staining of human colon cancers that uses these antibodies has demonstrated immunoreactivity in the invading tumor cells. Antibodies to type IV collagenase may serve as a marker for metastasis of the tumor cell and may be useful in development of new diagnostic procedures for identifying occult metastases.

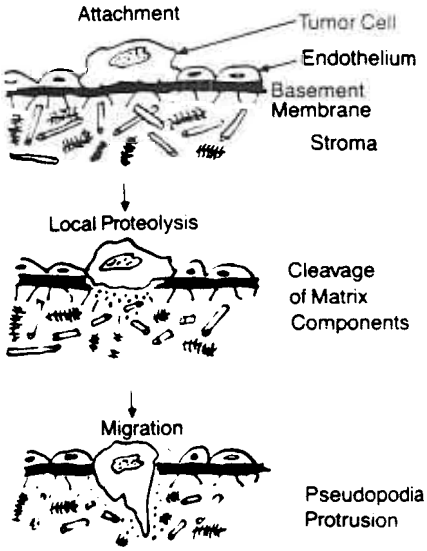

Fig 1.—Multistep metastatic cascade: tumor cells that arise from glandular epithelium penetrate the subepithelial basement membrane to gain access to stromal lymphatic vessels and blood vessels. Tumor cells attach to the basement membrane, ensheathing the capillaries and venules, and this is followed by invasion through the vessel wall to enter the circulation. Tumor cells are carried singly or in clumps in the blood, overcome host defenses, and arrest in the vascular bed of the target organ. The arrested tumor cells invade the endothelium and the basement membrane to exit the circulation and initiate a metastatic colony. As depicted herein, invasion of the basement membrane proceeds in two steps: (1) attachment, mediated by matrix receptors, and (2) local proteolysis, facilited by metalloproteinases, serine proteinases, and thiol proteinases.

Even if they can attach to and proteolyze the basement membrane through which they must pass, the tumor cells still fail to complete the invasion process without the ability to migrate, the third step of invasion. A new class of cytokines secreted by the tumor cell, the autocrine motility factors,[8] has been identified. These motility factors stimulate pseudopodial protrusion followed by random and directed locomotion by the tumor cells. These motility factors are produced by tumor cells in vivo, as demonstrated by experiments in which the content of motility factor was measured in the urine of patients with transitional cell carcinoma of the bladder.[17] Increasing content of the motility factor in a 24-hour urine collection was significantly related to the pathological grade of the bladder cancer. Patients with pathological grade 3 tumors, invading through the full thickness of the bladder, had a much higher content of the motility factor in their urine than the

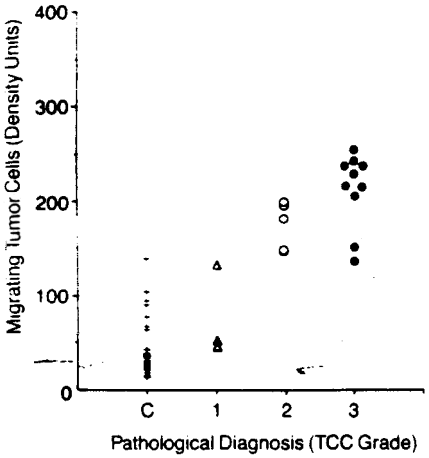

Fig 2.—Autocrine motility factor (AMF) content of human urine correlates with histological grade of transitional cell carcinoma (TCC) of the bladder.[17] Each data point represents the blinded study of an individual patient. The AMF was measured using motility assays; "density units" refers to densitometric analysis of the stained migrated cells in the motility assay. C indicates control cases; 1, grade 1, most differentiated; 2, grade 2; and 3, grade 3, least differentiated.

urine of normal patients or those with low-grade bladder carcinoma ($P<.001$) (Fig 2). The autocrine motility factor content of the urine was confirmed with immunoblotting as well as with enzyme-linked immunosorbant assay. Measurement of the motility factor in urine of patients who have bladder cancer may be an adjunct to pathological grading for the prediction of invasiveness.

Thus, there are several gene products that may correlate with the metastatic aggressiveness of tumors and may be necessary for a cell that is tumorigenic but nonmetastatic to become a cell that is fully metastatic. The metastatic phenotype may be the result of a complicated balance among production and response to growth factors, adhesion receptors, motility factors, proteases, and protease inhibitors.

What genetic events switch on this complicated cascade of factors necessary for the metastatic phenotype? A number of laboratories have transfected DNA from metastatic tumors into normal cells and nonmetastatic tumor cells in an attempt to induce them to become metastatic by switching on necessary gene products. The *ras* oncogene has a powerful effect on the metastatic phenotype.[18] Transfection of the *ras* oncogene into diploid rat embryo fibroblasts[11] caused the cells to become fully tumorigenic and highly metastatic. Furthermore, certain combinations of other oncogenes can lead to the tumorigenic and metastatic phenotype. Other combinations of oncogenes can suppress

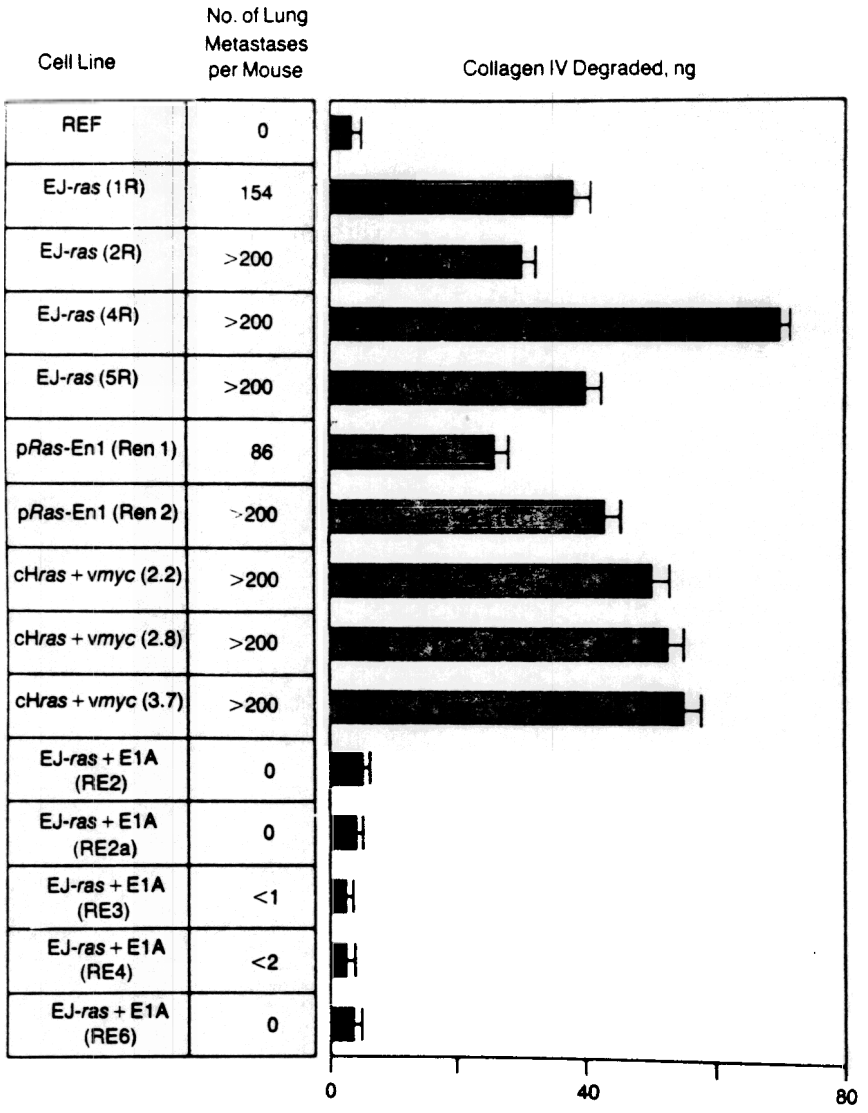

Fig 3.—Transfection of the *ras* oncogene into diploid rat embryo fibroblasts (REFs) induces tumorigenicity and metastasis. In contrast, transfection with *ras* plus adenovirus E1A suppresses metastatic phenotype, but the transformed cells remain fully tumorigenic. Only those clones that are metastatic express high levels of type IV collagenase, a basement membrane–degrading metalloprotease.[20] Horizontal bars indicate SEs.

the metastatic phenotype but do not affect the tumorigenic phenotype.[19] In this regard, the production of type IV collagenase has been compared with the metastatic potential in a series of rat embryo fibroblast clones transfected with *ras*, *ras* plus c-*myc*, or *ras* plus adenovirus E1A oncogenes. The oncogene *ras* plus E1A results in tumorigenic but totally nonmetastatic cells that fail to produce type IV collagenase, whereas the *ras* or *ras* plus c-*myc* transfectants produce high numbers of metastases and large amounts of type IV collagenase (Fig 3).[20] This demonstrates a biochemical link between type IV collagenase production and the metastatic phenotype. Based on these results, we can infer that certain human genes might be augmented in metastatic tumors, but others might be down regulated in association with metastases. This is the case in a series of experimental studies that use human tumors.

Selected oncogenes have been reported to be amplified in aggressive breast cancer; the presence of these oncogenes in the index patient's tumor might have suggested her ultimate progression. Amplified HER-2/*neu*, as reported by Slamon and coworkers,[21] was associated with increased numbers of lymph node metastases or a poorer prognosis. Lidereau et al[22] have shown recently that the amplified *int*-2 oncogene is associated with breast cancer aggressiveness. The mechanism by which these amplified genes relate to tumor aggressive-

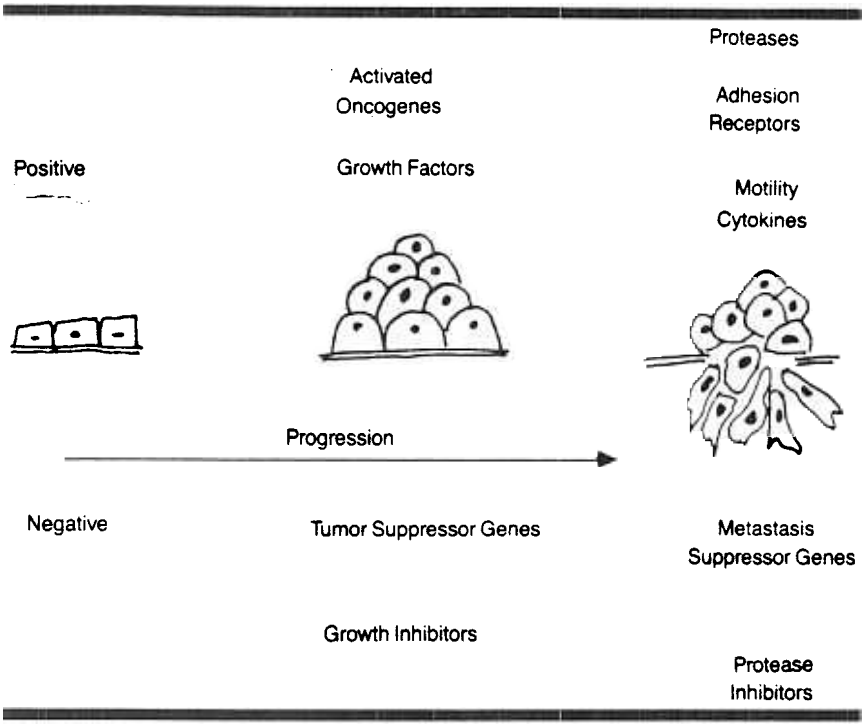

Fig 4.—Hypothetical sequence of cancer progression from benign to invasive metastatic disease. Activation of an oncogene is not sufficient to induce the invasive metastatic phenotype. Invasion and metastasis requires the further loss of genes that may inhibit the invasive phenotype.[25-27]

ness is unknown. Furthermore, experimental studies of human tumors have shown that certain chromosomal regions or genes may be lost in association with increased aggressiveness. As reported by Ali et al,[23] there may be deletions on chromosome 11p associated with breast tumors that relapse quickly. Vogelstein et al[24] have shown that progression of colonic polyps to colon cancer is associated with deletions on chromosomes 17 and 18.[25] A novel gene has been described, nm23, which is down regulated in its level of expression in metastatic murine melanomas and human breast cancers.[26,27] This gene may be one of many types of genes that normally suppress the metastatic phenotype, analogous to the experimental situation in which adenovirus E1A suppresses the metastatic phenotype.

Experimental and clinical evidence indicates that certain genes may be amplified or activated, and other genes may be down regulated or deleted, resulting in the metastatic phenotype. This hypothesis is depicted in Fig 4, which shows progression of colon cancer from an uninvolved mucosa to an invasive carcinoma to metastasis. During development of a premalignant polyp, activation of an oncogene may occur, which confers malignant tendencies. This may lead, if unrestrained by a suppressor gene, to development of a highly invasive tumor. However, if the suppressor gene is present, the activation of the oncogene may be necessary for tumorigenesis but not sufficient to lead to the metastatic phenotype, and additional steps will be required to obtain the full metastatic phenotype. There may be multiple positive and negative regulatory steps in the progression of tumor development that lead to metastases. Tumors may require different combinations of involved genes. Future work based on a detailed evaluation of this hypothesis will lead to new diagnostic and therapeutic strategies for combating cancer invasion and metastases.

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