Autocrine and Paracrine Growth Mechanisms in Cancer Progression and Metastasis

Garth L. Nicolson

The Institute for Molecular Medicine, Huntington Beach, California

GLOSSARY

autocrine growth factors Growth factors that are made by and that act on the same cell.

metastasis The spread of tumor cells via the lymph, blood, or body cavities to near or distant sites where new secondary tumors are formed.

paracrine growth factors Growth factors that are made and secreted by one cell and that act on adjacent cells in a tissue or organ.

tumor diversification Generation of heterogeneous subpopulations of tumor cells with differing phenotypes.

tumor instability Changes in tumor cell properties caused by irreversible modifications in the coding sequences of genes and by quantitative changes in gene expression.

tumor progression Sequential changes in tumorigenic and malignant properties of tumors that occur with time *in vivo*. The changes generally tend toward more malignant, dangerous states.

One of the most important characteristics of cancer cells is their ability to grow in unusual locations, especially at metastatic sites. The successful proliferation of cancer cells is due to their responses to local (paracrine) growth factors and inhibitors and their production and responses to their own (autocrine) growth factors. As tumors grow and evolve (tumor

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progression), they undergo changes in their growth and other properties. For example, when tumor cells invade and spread to other sites at the early stages of malignant tumor progression, there is a tendency for many common cancers to metastasize and grow preferentially at particular sites, suggesting that unique tissue paracrine growth mechanisms may dominate the growth signals processed by metastatic cells. At somewhat later stages of tumor progression, where widespread dissemination to various tissues and organs occurs, autocrine growth mechanisms may dominate. The progression of malignant cells to completely autonomous growth states can occur, and at this stage of tumor progression cell proliferation may be independent of growth factors or inhibitors.

I. INTRODUCTION

Most patients succumb to their metastatic disease, not their primary tumors; therefore, controlling the spread and growth of malignant cells at metastatic sites is an important challenge. Malignant tumors are characterized by differences in various properties, and those that are functionally involved in invasion and metastasis are among the most important properties of cancer cells that determine their survival and growth at secondary sites.

In addition to their invasive and metastatic properties, highly malignant cells are characterized by progressive changes in their genomes, particularly in genes that regulate and encode products important in certain phenotypic properties. Highly malignant cells are not particularly stable and they continually drift in their phenotypic properties. In some cases, such phenotypic drift in cancer cell properties is virtually undetectable, but in other cases it can be dramatic and result in obvious tumor cell heterogeneity. Advanced primary tumors that have not yet metastasized and cancers that have metastasized are made up of very dynamic, unstable cellular assemblages. The individual cancer cells that are unstable do not always undergo phenotypic drift. In some cases they interact with other tumor and normal cells that can stabilize the cell population as a whole and reduce the tendency for individual cells to diversify and become more variable in a variety of properties.

If malignant cells break loose from their primary site as individual cells or small groups of cells, some of these cells can diversify further and become even more heterogeneous in their properties. As stated earlier, this can occur because individual cells are modulated in their phenotypic properties by interactions with other cells. Once removed from these interactions, cancer cells undergo phenotypic diversification. Therefore, the invasion of individual cells away from the initial tumor site and into new tissue compartments can lead to cancer cells with differing properties from the primary tumors from which they were derived. However, most cellular properties do not change detectably as tumor cells in advanced primary tumors metastasize to distant sites. This may be due to the fact that advanced primary tumors have already undergone significant diversification and change.

Tumor cells progress to the malignant state and eventually the metastatic state in a process that is thought to occur by a stepwise series of genetic and epigenetic (nongenetic) changes. These changes occur apparently randomly, and accumulating enough changes necessary for a cancer cell to become highly metastatic can take years. Such metastatic cells were previously thought to be very rare cells that occasionally arose within the primary tumor, and it was thought that only these rare phenotypically stable cells were the cells capable of metastasizing. Although tumor progression can result in stepwise nonreversible changes and acquisition of a number of phenotypic properties that are important in the process of metastasis, it is now thought that tumor progression to the metastatic phenotype depends less on the selection of rare, stable metastatic cells than on the inherent instabilities of the tumor cells that comprise the primary neoplasm. This notion was advanced by Victor Ling, Ann Chambers, Richard Hill, and their colleagues at the Ontario Cancer Institute. They envisaged that malignant tumor cells are constantly undergoing rapid dynamic phenotypic changes in a process they called dynamic heterogeneity. Independently from the group in Canada, my colleagues and I at the University of Texas M. D. Anderson Cancer Center studied this phenomenon and termed it phenotypic drift. In either scheme individual cancer cells are thought to be changing constantly from the

metastatic to nonmetastatic phenotype and back to the metastatic phenotype. When the rate of change favors the appearance of metastatic cells, a tumor progresses to a more malignant phenotype capable of metastasizing.

Tumor phenotypic instability is probably due, in part, to essentially irreversible qualitative changes in gene structure (gene mutations, deletions, transpositions, amplifications, etc.) that can result in altered gene products and to dynamic quantitative changes in gene expression. The quantitative changes in gene expression result in transient changes in the amounts of various gene products in individual cancer cells. The net result is cellular variability within a primary tumor and eventually transient or, in some cases, even permanent acquisition of the metastatic phenotype by individual malignant cells. It is probably these unstable, highly malignant cells that ultimately give rise to tumor colonies at other sites. This could explain the problem in determining the particular molecular characteristics of metastatic cells. If metastatic cells possess unstable properties important in malignancy, these would be expected to be difficult to identify.

In contrast to the various unstable properties of metastatic cells, the proliferative properties required for survival and growth of metastatic cells at secondary sites must remain relatively stable if metastases grow to detectable sizes. Without stable tumor cell growth, only micrometastases would be present, and these would not be expected to kill the host. Thus an understanding of the process of malignant cell growth and its interference at metastatic sites may be important in the development of new therapies for restraining the growth of established metastases.

II. CANCER PROGRESSION AND PARACRINE GROWTH OF METASTATIC CELLS

As cancer progresses and individual cancer cells eventually acquire more malignant phenotypes, under the proper circumstances, they can invade and metastasize to near and distant sites. For many cancers this process is not random and cannot be explained by the anatomic site of the cancer or mechanical properties of cancer cells. Many cancers metastasize to sites unexpected on the basis of their circulatory or lymphatic connections or on their ability to mechanically lodge in the first lymph node or capillary bed encountered by cancer cells released from the primary cancer site. For example, the metastasis of cutaneous malignant melanoma to the brain but ocular malignant melanoma to the liver, prostate carcinoma to the bone, or colon carcinoma to the liver are examples of nonrandom metastatic spread. In addition, during tumor progression the evolving malignant cells can change in their tissue-metastatic properties. This observation has been made for a number of different histologic classes of cancer.

At the initial stages of metastasis, many possess a tendency to metastasize to particular sites. This so-called organ specificity or organ preference of metastasis occurs at early stages of metastatic progression, but at later stages of progression where metastasis is widespread and many secondary sites are involved, more organs and tissues are colonized by metastatic cells. Strictly speaking, the site specificity of cancer metastasis is usually only a *preference* for colonizing certain sites; it is not entirely a site-specific process, except for very few cancers. Nonetheless, it is an important phenomenon that appears to be based on the unique properties of the cancer cells as well as the host microenvironments. This notion was first advanced over 100 years ago by Stephen Paget, who was treating breast cancer patients in London. Paget advanced the "seed" and "soil" hypothesis that individual cancer cells or seeds can only grow in suitable soil. In terms of their growth properties, neoplastic cells that progress to the metastatic phenotype and colonize distant sites should be less dependent on their usual growth signals and more growth responsive to growth signals at their new metastatic sites.

The increased responsiveness of some cancer cells to paracrine growth factors or decreased responsiveness to paracrine growth inhibitors expressed differentially at particular metastatic sites could explain why certain cancers show a preference for metastatic growth at certain sites. This could also explain the finding of increased numbers or affinities of particular growth factor receptors and enhanced responses to certain growth factors. Overexpression of specific growth factor receptors correlates with progression

and metastasis of certain cancers. For example, the epidermal growth factor (EGF) receptor is often associated with poor prognosis or enhanced metastasis in breast, lung, and bladder cancers and melanomas. In some cases, overexpression of a oncogene, such as c-*erb*B-2/*neu*-encoded putative growth factor receptor, is associated with poor prognosis of breast and ovarian carcinomas. Other examples of alterations exist in growth factor receptors, but in general, those growth factor receptors that are probably important in metastasis are usually the ones that change in their expression in highly metastatic cancers.

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The overexpression of growth factor receptors has been accomplished experimentally by gene transfer techniques, and the biological properties of the recipient cells can be tested in suitable animal hosts. For example, in my department at the University of Texas M. D. Anderson Cancer Center, Dihua Yu, Mienchie Hung, and I found that the transfer of a mutated c-*erb*B-2/*neu* oncogene into benign cells resulted in conversion to the metastatic phenotype. Although the changes in metastatic properties and enhanced growth potential of the growth factor receptor gene-transferred cells can be explained by oncogene transfer, this experimental result is not always found. Changes in the metastatic properties of c-*erb*B-2/*neu* gene-transferred cells occurred concomitant with changes in several metastasis-associated properties, including increased adhesion to microvessel endothelial cells, particularly endothelial cells derived from the target organs for metastasis, increased invasiveness of extracellular matrix and reconstituted basement membrane matrix, increased cell motility in response to organ-derived chemotactic factors that stimulate directed tumor cell invasion, and increased responses to organ-derived paracrine growth factors. In these experiments we found that the oncogene-mediated conversion of benign tumor cells to metastatic cells was accompanied by changes in growth factor receptor expression and growth factor responses as well as by changes in the expression of other gene products involved in various steps of the metastatic process.

To test the hypothesis that organ growth properties are important in organ preference of metastasis, we found that the organ preference of metastatic cell growth, at least at the initial stages of metastatic pro-

gression, was related to the differential responses of metastatic cells to paracrine growth factors and inhibitors secreted by the target organ tissues. Therefore, differentially expressed paracrine growth factors and inhibitors in different organs and tissues probably determine, to some degree, the growth potentials of cancer cells at metastatic sites. Thus cancers, at least at their initial stages, should be dependent on paracrine growth factors released from surrounding normal cells. After progression to the metastatic phenotype, growth factor responses are often changed, and they should be more compatible with the responses to cytokines expressed at secondary metastatic sites.

Tumor models have been used to demonstrate that the organ preference of metastasis is related to enhanced growth responses mediated by cytokines, growth factors, and inhibitors released at secondary organ sites. Using lung- and ovary-colonizing murine melanoma sublines and liver- and lung-metastasizing large cell lymphoma cell lines, we demonstrated that tumor cell growth in serum-limited culture medium was differentially stimulated by soluble factors released from different organ tissues. In these examples, lung- and liver-metastatic tumor cells were growth stimulated better by factors released from lung and liver tissue, respectively. In contrast, other tissue-conditioned media inhibited or had no effect on tumor cell growth.

Tissue-derived growth-promoting substances have been identified and partially purified from the culture medium conditioned by certain organ tissues. For example, we purified to homogeneity a lung-derived metastatic cell growth factor from lung-conditioned medium, and from its amino acid sequence we were able to identify the growth factor as a transferrin. Transferrins are iron-transferring ferroproteins that are required for cell growth. Some years ago Pedro Cuatracasas found that transferrins are more than iron transport proteins and that they have mitogenic properties beyond their nutrient transport. The tissue-derived transferrins that we isolated are probably used as paracrine growth stimulators in several tissues. The transferrin isolated from lung tissue-conditioned medium was the first tumor cell growth factor purified to homogeneity on the basis of its ability to differentially stimulate the growth of highly

metastatic cells. We examined a number of different tumor metastatic systems and found that several other metastatic cell lines were more responsive to transferrin. To demonstrate that a transferrin-like activity in tissue-conditioned medium is responsible, in part, for the stimulation of metastatic cell growth, transferrin-like molecules were removed from tissue-conditioned medium. After the removal of transferrin molecules, most of the growth properties of the organ tissue-conditioned medium were lost.

Other organ compartments are also important sites of metastatic involvement and apparently have their own set of important cytokines. For example, bone is an important metastatic site for prostate cancer, and transferrin was found to be a major growth factor for bone-metastasizing prostatic carcinoma cells. How are transferrins used as specific organ cytokines if they are found at several organ sites? The answer may be that the relative concentrations of the transferrins and other cytokines in different tissues are different. Using a series of melanoma cell lines of differing metastatic potentials to sites such as brain, we found that brain- and lung-colonizing melanoma lines responded best to the lowest concentrations of transferrins and expressed the highest numbers of transferrin receptors. Transferrin receptor numbers were highest in brain metastatic lines and decreased in the following order: high brain-metastasizing ability > high lung-metastasizing ability > intermediate lung-metastasizing ability > poor metastatic capability. Thus, a hierarchy of transferrin expression exists in different organs and may be important in determining metastatic cell growth. Cancer cells with greater numbers of transferrin receptors (or different affinities) may be more successful at growing at sites that express low concentrations of transferrin molecules.

Brain is an example of an organ that is not particularly susceptible to metastatic colonization. Brain metastases are rarely produced by cancers, but in some malignancies, such as melanoma and breast cancer, brain metastases are quite commonly found. For malignant cells to metastasize to brain, it may be advantageous for them to express high numbers of particular growth factor receptors, such as transferrin receptors, and respond to low concentrations of growth factors, such as the transferrins. Further support for the evolution of enhanced transferrin responsiveness and the metastasis of various tumor cells comes from the selection of high transferrin receptor-expressing variants from poorly metastatic cells. The high transferrin receptor-expressing cells displayed increased spontaneous metastatic properties and grew faster compared to low transferrin receptor-expressing cells. Brain-metastasizing cancer cells appear to also respond to other paracrine growth factors at secondary sites, and it is likely that paracrine sources of transferrin provide only one of several growth factors important in determining the organ preference of metastatic cell growth. Differences in the concentrations of various cytokines, growth factors, and inhibitors may be important in providing the correct environment for metastatic cell growth.

The normal functions of paracrine growth factors are not known, but they might be involved in controlling cell growth and local tissue regeneration during wounding and inflammation. Metastases often occur at the sites of trauma or tissue damage. Thus the normal role of the paracrine growth factors and growth inhibitors is probably in organ repair.

Another source of organ-derived growth-promoting molecules are extracellular matrix, tissue stroma, and basement membranes. Extracellular matrix and basement membranes contain tightly bound growth factors that can be released by tumor cell degradative enzymes and stimulate tumor cell growth. Extracellular matrix molecules themselves may modulate tumor cell growth and the state of tumor cell differentiation. For example, the maintenance of normal breast cells is dependent on lactogenic hormones and extracellular matrix, and matrix molecules can regulate gene expression and growth of particular normal cells. Metastatic cells often show differential growth responses to extracellular matrix molecules. Using extracellular matrix obtained from several organs, Lola Reid and collaborators at the Albert Einstein Medical Center found that metastatic mammary carcinoma and hepatoma cells were differentially stimulated to grow at low cell densities by organ matrix isolated from the target organs for metastasis formation. However, they did not find the same pattern of growth stimulation if metastatic cells were plated at high cell densities on the various extracellular matrices, suggesting that extracellular matrix growth stimulatory molecules are more important at the early

stages of cancer cell growth, such as in micrometastases, rather than at the later stages of cell growth at high cell densities, such as would be expected in gross clinically detectable metastases. Thus the unique growth microenvironments for cancer cells in various organs and tissues are probably determined collectively by tumor cell responses to cell-bound, matrix-bound, and soluble paracrine factors.

III. PARACRINE GROWTH INHIBITORS OF METASTATIC CELLS

Cancer cells also receive and process paracrine negative growth signals. Only a few organ-derived paracrine growth inhibitory molecules have been identified. In most cases these have turned out to be well-known cytokines, such as the transforming growth factor-β (TGF-β) family. Certain organ cells can release potent growth inhibitors that prevent the growth of malignant cells, and these factors could be important in determining metastatic cell growth at particular sites. For example, kidney cell-conditioned medium is particularly inhibitory for many cancer cells, and most cancers and metastatic model systems fail to metastasize to the kidney. The most potent growth inhibitor released by kidney tissue has been identified as TGF-β1. TGF-β1 can inhibit the growth of several highly metastatic cell lines. Not all metastatic cells are growth inhibited by TGF-β, but this family of cytokines is very important in cancer metastasis. An interesting finding is the growth stimulation of metastatic cells by TGF-β and the inhibition of growth of tumor cells from primary sites, but this does not appear to be a general phenomenon.

The growth responses of malignant cells can change during progression to more malignant phenotypes. For example, the growth responses of early lateral growth phase human melanoma cells and more advanced vertical growth phase melanoma cells have been studied by Robert Kerbel and Meenhard Herlyn. Only the vertical growth phase melanomas are dangerous, and patients with these lesions are at risk to develop metastases. Studies on the responses of melanoma cells to positive and negative growth cytokines indicated that the more progressed melanoma cells lose responsiveness to negative growth inhibitors.

The molecule responsible for differentially inhibiting the growth of early lateral growth phase melanoma cells was purified and shown to be interleukin-6 (IL-6), a well-known hemopoietic cytokine. This cytokine is produced by a variety of tissues, among them keratinocytes, endothelial cells, fibroblasts, macrophages, and monocytes. The inhibitory responses to recombinant IL-6 were not duplicated with more advanced vertical growth phase melanoma cells. It was subsequently established that the more highly progressed metastatic cells uniformly lost responsiveness to a variety of growth inhibitors. Loss of paracrine inhibitor responses could be as important to the formation of metastasis as changes in paracrine growth factor responsiveness.

IV. SOURCES OF PARACRINE GROWTH FACTORS AND INHIBITORS

The sources of paracrine growth factors and inhibitors in various organ tissues are largely undetermined. Parenchymal cells, fibroblasts, endothelial cells, mast cells, and macrophages, among other cell types, and factors from acellular sources, including interstitial extracellular matrix and basement membranes, could collectively provide various growth factors and inhibitors. Some of the growth factors expressed by parenchymal cells are released as soluble molecules, whereas some are not released and require cell–cell or cell–matrix contact. When lung- and liver-colonizing malignant melanoma cells were cocultured with normal hepatocytes, Max Burger and colleagues found that only liver-colonizing melanoma cells were growth stimulated. The stimulation required cell-to-cell contact with the hepatocytes and was not duplicated with liver tissue-conditioned medium. Thus in some metastatic systems, organ parenchymal cells are important sources of tumor cell growth stimulation.

Microvascular endothelial cells are important in metastatic cell growth. Using lung- and liver-colonizing large cell lymphoma cells, we showed that conditioned medium from organ-derived microvessel endothelial cells could substitute for organ tissue-conditioned medium in tumor cell proliferation as-

says. Interestingly, liver-colonizing large cell lymphoma cells responded best to conditioned medium from liver sinusoidal endothelial cells, whereas lung-colonizing lymphoma cells responded best to conditioned medium from lung microvessel endothelial cells. Removal of transferrin from the lung endothelial cell-conditioned medium resulted in a reduction of mitogenic activity, but some activity remained that was not associated with transferrin. Endothelial cells can respond, in turn, to growth and motility factors, called angiogenesis factors, released by malignant cells. A reciprocal relationship may exist between tumor cells and specific organ-derived normal cells (Fig. 1). This relationship extends to other cell types as well as to extracellular matrix. Thus malignant cells can stimulate as well as be stimulated by normal host cells.

Fibroblasts isolated from different tissues have been used to differentially stimulate the growth of cancer cells. Using lateral and vertical growth phase melanoma cells, the growth responses of these cells to fibroblasts from various tissue sources have been tested in cocultures. Fibroblasts isolated from dermal tissue generally inhibited the growth of early lateral growth phase human melanoma cells but had stimulatory or little effect on more advanced vertical growth phase melanoma cells. In this case, the inhibitory molecule

FIGURE 1   Reciprocal interactions between malignant cells and their cellular microenvironments. Cancer cells release paracrine factors that can affect host cells, such as parenchymal cells, endothelial cells, fibroblasts, mast cells, granulocytes, macrophages, and tissue extracellular matrix. In turn, the host cells and matrix can release paracrine factors that stimulate or inhibit tumor cell proliferation. Tumor cells can also synthesize autocrine factors that can act inside the cell (private or intracellular) or outside the cell (public or extracellular).

responsible was identified as IL-6 released by dermal fibroblasts. We also found that metastatic and non-metastatic mammary carcinoma cells responded differentially to tissue-derived fibroblast-conditioned medium, and this was related to the organ preference of metastasis. The highest growth stimulation was from fibroblasts isolated from lung and mammary gland, targets for metastatic cell growth in this system. The fibroblast-derived growth factor was not related to transferrin because we demonstrated that the organ-derived fibroblasts did not synthesize detectable amounts of transferrin. Peter Jones has examined the growth properties of bladder carcinomas of different grade and invasive properties. Using bladder carcinomas of differing grade, differentiation, and invasive properties, only the poorly differentiated high-grade, invasive bladder carcinoma cells were growth stimulated by bladder-derived fibroblasts. When prostate carcinoma cells were cocultured with fibroblasts from different tissue sources, an interesting relationship was found. Similar to the reciprocal relationship between cancer cells and endothelial cells, bidirectional stimulation of growth was seen by Lelung Chung when conditioned medium from prostate carcinoma cells was tested with conditioned medium from bone fibroblasts. Because bone is a common site of prostate metastases, this suggested that prostate carcinoma cells stimulate and are stimulated by target bone fibroblasts. This reciprocal or bilateral relationship between metastatic cells and host cells in the target site for metastasis appears to be an important feature of metastatic cell colonization (Fig. 2).

Mast cells are another source of mitogens and motility factors for metastatic cells. Similar to the process of inflammation, mast cells are attracted to tumor sites by the release of mast cell mediators. Mast cells often associate at the periphery of tumors and can release tumor cell mitogens and motility factors that can differentially affect the growth and motility of malignant cells. With Mustafa Dabbous we found that only highly metastatic mammary cells attracted large numbers of mast cells into the tumor periphery. Mast cells isolated from the tumor periphery were found to release factors that differentially stimulated the growth of the highly metastatic but not poorly metastatic cells. A function for the mast cell mitogens was shown *in vivo* by administering drugs that are
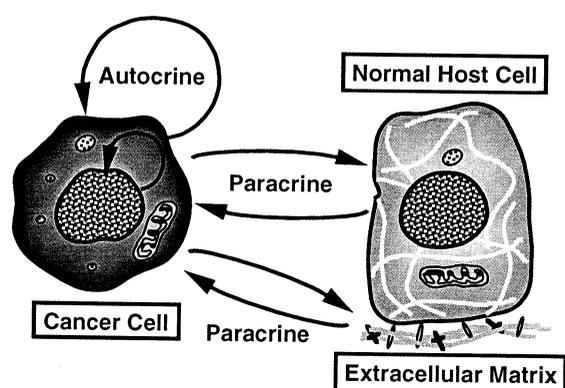
Platelets
- Growth Factors
- Coagulation Factors
- Enzymes

T Cells
- Cytokines
- Cytolytic Factors
- Degradative Enzymes

NK & LAK Cells
- Cytokines
- Cytolytic Factors
- Degradative Enzymes

Endothelial Cells
- Growth Factors
- Growth Inhibitors
- Fibrinolytic Factors
- Enzymes, Nutrients

Macrophages
- Cytolytic Factors
- Cytostatic Factors
- Mutagenic Factors
- Growth Factors

Matrix

Mast Cells
- Growth Factors
- Glycosaminoglycans
- Extracellular Matrix
- Cytokines, Histamine
- Degradative Enzymes

Tumor Cells
- Cytolic Factors
- Chemotactic Factors
- Degradative Enzymes
- Procoagulants, MIFs
- Secreted Antigens

Fibroblasts
- Growth Factors
- Differentiation Factors
- Inductive Factors
- Other Cytokines
- Extracellular Matrix
- Degradative Enzymes
- Enzyme Inhibitors

Parenchymal Cells
- Growth Factors
- Growth Inhibitors
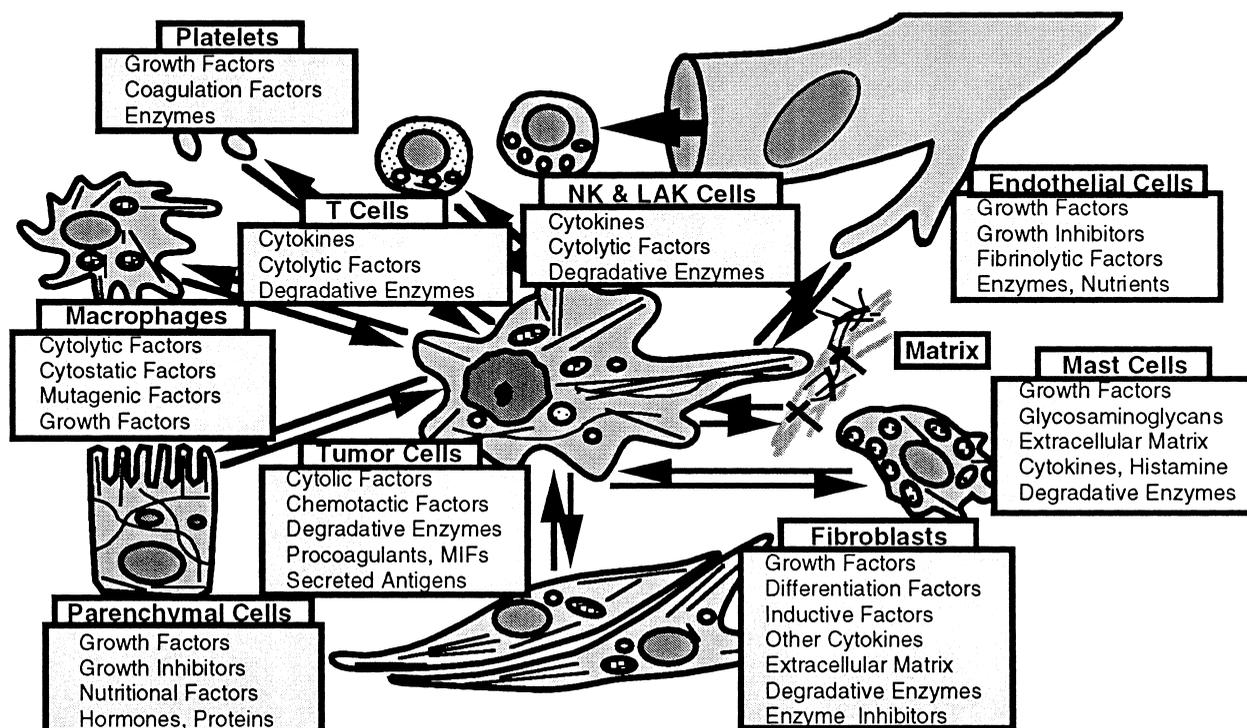- Nutritional Factors
- Hormones, Proteins

FIGURE 2　Tumor microenvironment and the role of paracrine cytokines in regulating tumor cell growth, differentiation, and malignant properties. Reciprocal or bidirectional interactions between various cells in the tumor microenvironment are important in the establishment of the tumor microenvironment.

mast cell stabilizers to animals receiving metastatic cell implants. Mast cell stabilizers prevented the release of mast cell contents and reduced the growth and metastases of the highly metastatic cells growing at their normal organ sites.

V. AUTOCRINE GROWTH MECHANISMS IN CANCER METASTASIS

Cancer cells have the capacity to synthesize and release multiple growth factors that can act on the tumor cells themselves in tumor cell autocrine loop mechanisms. This can occur either by extracellular release of the growth factor and its binding to an appropriate extracellular receptor on the same cell (public or extracellular autocrine mechanism) or by ligand–receptor interaction inside the cell (private or intracellular autocrine mechanism, Fig. 1). When highly malignant and metastatic cells are examined for the synthesis of autocrine growth factors, they are

commonly found to make and use a variety of growth factors. Interestingly, these are often the same growth factors that normally stimulate the growth of normal cells from which the tumor cells were derived.

Autocrine growth factors play an important role in neoplastic transformation, but their role in cancer metastasis is somewhat less clear. Studies on tumor cells derived from primary and metastatic sites or sequential selection of malignant or metastatic variants have yielded important but inconclusive data on the role of autocrine growth factors in metastasis. As tumors progress to more malignant or metastatic phenotypes, they generally become less dependent on serum-derived growth factors for their growth and they begin to produce polypeptide growth factors, suggesting that autocrine growth mechanisms may be involved in metastasis formation. There are, however, examples where the autocrine production of a growth factor by malignant cells did not correlate with tumor progression or stage. For example, the production and secretion of melanoma growth-stimulating activity by melanoma cells and bombesin-like (gastrin-releasing

peptide) activity by small cell lung carcinoma cells were not related to tumor stage or tumor progression. The usual result is a loss of growth factor responsiveness with tumor progression to the metastatic phenotype. For example, Meenhard Herlyn and collaborators found that cell lines established from dysplastic nevi had similar growth factor requirements to normal melanocytes, whereas malignant melanoma cell lines had reduced requirements for a variety of growth factors. Only cell lines established from melanoma metastases could be quickly adapted to *in vitro* growth in serum-free medium. When they sequentially selected human melanoma cell lines that were established from a primary melanoma lesion for their ability to invade a reconstituted basement membrane, they showed that only the most invasive tumor cell variants were spontaneously metastatic in nude mice and that these same cell lines were less serum dependent for growth in tissue culture. Selection for growth in serum-free medium resulted in increases in invasive and metastatic properties, but the selected melanoma cell lines were unstable and in the absence of continued selective pressure reverted back to the phenotype of the parental cell line. The relative instability of metastasis-associated properties was mentioned earlier, and transient changes in gene expression are expected when a strong selective pressure is removed from a population of inherently unstable malignant cells.

The release of autocrine cytokines can also produce effects on neighboring cancer cells. The release of a cytokine from one tumor cell and its effect on neighboring cells is a form of paracrine signaling among tumor cell populations that may be important in modifying the properties of the tumor. In a few cases, the clonal effects of tumor cells on surrounding tumor cells have been examined and found to affect tumor cell properties, especially malignant cell properties. When we examined the interclonal interactions of a series of melanoma cell lines, we found that the expression and the display of a cell surface glycoprotein that later was identified as a growth factor receptor were affected by clonal cell interactions along with metastatic properties. Here the interclonal tumor cell interactions stabilized the phenotypic properties of the malignant cells, but when the malignant cells were grown separately, they quickly lost their metastatic and organ growth properties. Thus tumor cell–cell

and host cell–tumor cell interactions are important in the metastasis and growth of malignant cells.

In some tumor systems, both paracrine and autocrine signals are important in metastasis. For example, hepatocyte growth factor, which is also known as scatter factor (HGF/SF), plays autocrine and paracine roles in both cell motility and growth. The HGF/SF receptor at the cell surface is encoded by the oncogene *met* or its normal cell counterpart, c-*met*. HGF/SF has been shown to be synthesized by a number of mesenchymal cell types, and it can act as a paracrine stimulator of metastasis at specific organ sites, such as liver and lung. Certain melanoma cells selected for liver-specific metastasis overexpress the HGF/SF receptor, and exposure of these cells to HGF augments cell motility and invasive behavior. Upregulation of c-*met* in these cells and its HGF/SF receptor by various methods increases liver colonization ability but does not change the organ metastatic specificities of these cells. Treatment of certain mammary adenocarcinoma cells with HGF stimulates their ability to metastasize to lung.

VI. GENE TRANSFER AND METASTATIC CELL GROWTH PROPERTIES

Transfer of a growth factor gene or a growth factor receptor gene into suitable untransformed recipient cells can result in these cells acquiring malignant growth characteristics. For example, we found that transfection of the transferrin receptor gene resulted in an increased ability to grow in serum-free medium with transferrin as the sole supplement along with acquisition of the metastatic phenotype. In other experiments, transfer of the oncogene c-*sis* that encodes the platelet-derived growth factor (PDGF) receptor, c-*erb*B-1 that encodes the EGF receptor, or c-*erb*B-2/*neu* that encodes a growth factor-related receptor by use of viruses increases the growth potential of the gene-transferred cells, resulting in oncogenic transformation. In some cases, however, the transfer of a growth factor gene does not result in neoplastic transformation unless the encoded growth factor is synthesized in a form that can be cell secreted and bind to a cell surface receptor. For example, Michael Klagsbrun found that the addition of basic fibroblast growth

factor (bFGF) by itself or transfer of the bFGF gene alone was not transforming to recipient cells. However, when the bFGF gene was fused to a secretion signal sequence to facilitate bFGF secretion, only the chimeric signal peptide–bFGF gene was transforming. In this example, the appropriate location of the growth factor was important to its ability to signal growth. In addition to tumor cell growth, FGFs are also important in endothelial cell proliferation and angiogenesis.

Using gene transfer techniques to stimulate cells to secrete growth factors and become transformed in the process assumes that the technique itself does not cause other changes in the recipient cell. This turns out to be important because highly malignant cells are also unstable, and the gene transfer techniques can destabilize cells in a process that leads to cellular instability, diversification, and cellular heterogeneity. Controversy exists as to whether additional genomic changes are required for the transformation of cells that have received growth factor genes. An example of this is the use of transforming sequence containing a gene for one of the PDGF polypeptide chains. The PDGF molecule is made up of two polypeptide (A and B) chains. Only the B chain gene was found to have a transforming sequence in at least two retroviruses, a class of transforming virus that has a propensity to pick up critical host gene sequences that can transform normal cells to neoplastic cells. The PDGF receptor homologue that constitutes the c-*sis* retroviral gene is oncogenic, suggesting the importance of growth factor genes and their receptors in neoplastic transformation. Although overexpression of the c-*sis* oncogene can result in neoplastic transformation, the addition of excess PDGF to untransformed cells does not reproduce this phenomenon. This apparent paradox can be explained by considering that the increased rate of cell proliferation caused by PDGF probably results in expansion of a subpopulation of preneoplastic cells that are potential targets for oncogenic transformation. Alternatively, the insertion of the growth factor gene itself causes mutation of cellular genes or other changes that are necessary for neoplastic transformation.

Gene transfer techniques have been used to test for the involvement of autocrine growth mechanisms in metastasis formation. Transfection of human breast cancer cells with the HGF/SF gene establishes an autocrine loop, and these cells demonstrate a greater propensity to metastasize to the lung. Cotransfection of murine 3T3 cells with genes encoding HGF/SF and its receptor results in cells that acquire the metastatic phenotype, especially lung-metastatic ability. Arnold Greenberg and collaborators used a transforming chimeric bFGF gene construct to transfect untransformed but highly unstable preneoplastic ells. The signal peptide–bFGF chimeric gene-transfected cells formed experimental metastases, whereas the bFGF gene-transfected cells without the signal peptide did not. In addition to experiments where untransformed cells were converted to malignancy by transfer of a gene encoding a growth factor that the recipient cells can respond to, cells have been transfected with growth factor genes encoding factors that they normally cannot respond to. For example, untransformed NIH-3T3 cells are not normally responsive to colony-stimulating factor-1 (CSF-1) because they lack the CSF-1 receptor. Using v-*fms*-transfected cells (v-*fms* is an oncogene encoding a CSF-1-like receptor), Greenberg and collaborators found that an addition of exogenous CSF-1 stimulated cell growth. *In vivo* the v-*fms*-transformed cells formed experimental metastases, whereas the untransformed cells did not. The addition of exogenous CSF-1 to v-*fms*-transformed cells before injection into animals resulted in greater numbers of experimental metastases in mice. However, if v-*fms*-transformed cells were allowed to grow under culture conditions where autocrine growth factor-conditioning could occur, and then the cells were treated with CSF-1, the opposite effect was obtained and the v-*fms*-transformed cells formed fewer metastases. This inhibitory effect was probably due to downregulation of the CSF-1 receptors on the v-*fms*-transfected cells by excess exogenous CSF-1. The differing effects of growth factors on the transfected NIH-3T3 cells were explained as being due to receptor occupancy and growth factor saturation effects. Although the levels of secreted autocrine growth factors, their receptors, and receptor occupancy were not determined, the saturation of growth factor receptors by exogenous growth factors could cause effects other than growth stimulation, such as growth inhibition or differentiation. Different concentrations of certain growth factors can have op-

posite effects on the same cell system. Often low concentrations of cytokines and growth factors can be stimulatory, whereas high concentrations of the same factor can be inhibitory. In addition, as discussed earlier, the transfer of genes into unstable cells can result in cellular diversification and heterogeneity.

Another example of loss of responsiveness with tumor progression is the loss of hormone responses with progression of breast cancer. Loss in estrogen dependency of MCF-7 breast cancer cells after transfection with the v-H-*ras* oncogene has been seen by Marc Lippman and collaborators. In contrast to parental MCF-7 cells, *ras*-transfected MCF-7 cells were tumorigenic in the absence of infused estrogens, and the transfected cells secreted TGF-α, TGF-β, and insulin-like growth factor-I, without a change in their cell surface growth factor receptor densities. Because advanced breast cancers often become refractory to estrogens and other hormones, this suggests that as malignant cells become more advanced, they become less dependent on systemic hormones and they possibly secrete higher amounts of autocrine growth factors. In support of this notion is the loss of hormone responsiveness of breast cancers as they progress *in vivo*. Breast cancers that are initially responsive to 17β-estradiol lose their hormone responsiveness as they progress to more malignant and aggressive, hormone-independent phenotypes. They can also increase their synthesis and secretion of autocrine growth factors. These finding are consistent with a generalized loss of growth factor regulation and an increase in autocrine growth mechanisms with progression to more malignant cellular phenotypes.

VII. NEW APPROACHES TO CANCER THERAPY

The information on growth requirements, expression of growth factor receptors, and growth inhibitor receptors by malignant cells should be useful in developing new therapeutic approaches to limiting the growth of metastatic cancers. Specific approaches used to limit the proliferation of tumor cells include the administration of growth inhibitors or analogs of growth factors or the use of antibodies against growth factor receptors to deliver toxins to highly metastatic

cells. For example, John Mulshine and collaborators found that a bombesin/gastrin-releasing peptide is a commonly found autocrine growth factor made by small cell lung cancer cells, and bombesin analogs have been used to inhibit the growth of the lung carcinoma cells *in vitro* in clonogenic growth assays and *in vivo* in xenographs in nude mice. Alternatively, to inhibit autocrine/paracrine growth pathways, antibodies have been administered that bind to the growth factors and remove or prevent them from interacting with tumor cells. For example, bombesin/gastrin-releasing peptides can be eliminated by administering monoclonal antibodies against these factors. A phase I clinical trial using a monoclonal antibody against a bombesin/gastrin-releasing peptide indicated that the effects of the bombesin/gastrin-releasing peptide can be partially blocked without apparent toxicity.

The employment of antibodies against growth factor receptors has been effective in preventing tumor growth. John Mendelson and collaborators and Ralph Reisfeld independently were among the first to use monoclonal antibodies against the EGF receptor to inhibit the growth of human tumors in nude mice. One of the monoclonal antibodies that Mendelson and colleagues used was also found to activate macrophage- and complement-dependent lysis of cancer cells *in vitro*, suggesting that the effects of growth factor receptor antibodies on the growth of tumor cells *in vivo* could be due, in part, to blocking host responses against the tumor. The formation of metastases from implants of human melanoma cells in severely immunodeficient mice has been blocked with a monoclonal antibody against the EGF receptor. When the antibody Fc or tail portion was removed, the tailless monoclonal antibody did not inhibit metastasis, suggesting that the Fc or tail portion of the antibody contributed to the anti-metastatic effect, possibly by an antibody-dependent host cell effector mechanism.

Various investigators have coupled the antibodies to toxins or toxin subunits to increase the effectiveness of antigrowth factor receptor monoclonal antibodies in suppressing the growth and metastasis of human tumors. Using extremely toxic molecules covalently bound to the targeting monoclonal antibody, specific killing of malignant cells has been achieved. The chimeric toxin–antibody molecules

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can produce antitumor effects without apparent side effects and suppression of white blood cells, even though these normal cells also express the growth factor receptors. Although it remains to be demonstrated that every last malignant cell can be killed with such toxin–antibody conjugates, the use of potent toxin conjugates to specifically kill metastatic cells at secondary sites may be an achievable goal that is well within our technical ability.

VIII. GROWTH RESPONSES OF METASTATIC CELLS AND MALIGNANT PROGRESSION

During the progression of malignant tumors there is often a tendency for the most malignant cells in a tumor cell population to lose expression of growth factor or inhibitor receptors and lose responsiveness to particular growth factors or inhibitors. The loss of these responses in highly metastatic cancer cells and the ability of such cancers to colonize and grow at distinct secondary sites may be explainable by considering each stage of cancer progression. At the early stages of metastasis, many cancers show restricted or-

gan preference of metastatis, whereas at the final stages near host death, these same cancers often colonize multiple organ and tissues sites. The explanation for this is that cancers progress from mainly paracrine growth stimulatory and inhibitory mechanisms at the initial stages of metastatic progression to mainly autocrine stimulatory mechanisms at the final terminal stages (Fig. 3). As discussed earlier, highly advanced cancers can secrete a variety of growth factors that could serve as autocrine sources of growth stimulation independent of their microenvironments. This could explain the loss of organ preference of metastasis seen at the later stages of cancer progression, whereas at earlier stages of cancer progression fewer organs are usually involved. Eventually metastatic progression continues and alterations in tumor cells may produce what has been termed an acrine (lack of regulation) state where malignant cells have lost their usual growth factor and inhibitor responses and are refractory to growth regulation by cytokines and growth factors and inhibitors. Because acrine malignant cells are not expected to respond to endogenous or exogenous growth factors or inhibitors, they should be the ultimate autonomous cells.
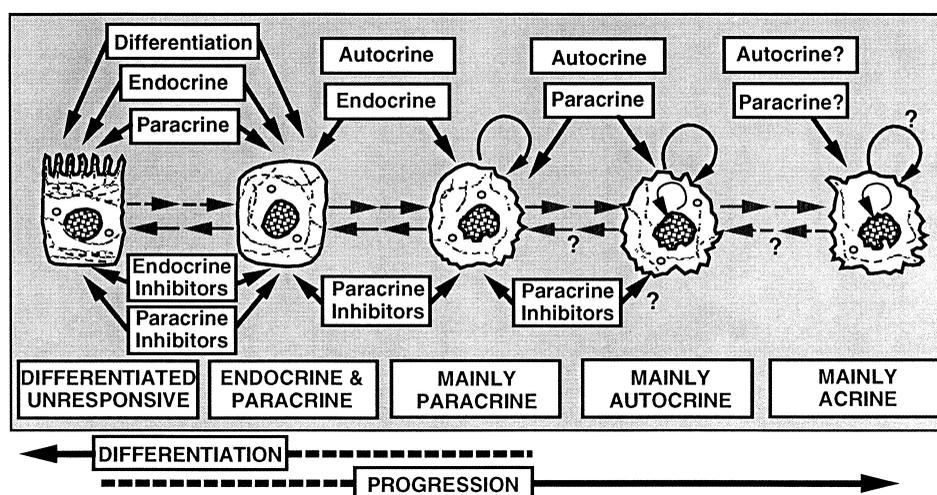
The concept that growth stimulatory and inhibitory

FIGURE 3 Progression of malignant cancer cells results in alterations in their responsiveness to host-derived growth stimulatory and inhibitory molecules and synthesis of autocrine growth factors. Tumor cell differentiation is shown as a possible process to the left and tumor cell progression to the right. Tumor progression can result initially in an increase in paracrine growth factor responses, as in the case of organ preference of metastatic colonization. As tumor progression continues, however, loss of paracrine growth factor and growth inhibitor responsiveness occurs and increases in autocrine growth factor production occur with more widespread tissue metastatic colonization. Eventually, tumor progression can result in a complete loss of growth factor/inhibitor responses (acrine state) and metastatic colonization of virtually any tissue can occur.

responses are altered during cancer progression has important significance for the development of new therapies for metastatic cancers. If highly progressed metastatic cells are more refractory to growth regulation, then it is unlikely that therapeutic intervention using analogs of growth inhibitors, monoclonal antibodies against growth factors or their receptors, or other means of suppressing growth-stimulating molecules or enhancing growth-inhibitory molecules will be useful in highly advanced cancers. Because these highly progressed cancers are often unstable and produce cell progeny that are unstable and undergo rapid changes in their gene expression programs, it is unlikely that therapies based on growth properties of malignant cells will succeed at the terminal stages of cancer progression. Their use must be at the earliest stages of cancer progression and metastasis, where malignant cells are still responsive to growth signals.

See Also the Following Articles

HEMATOPOIETIC GROWTH FACTORS • MOLECULAR MECHANISMS OF CANCER INVASION • TUMOR CELL MOTILITY AND INVASION

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