Cancer is a complex of many types of malignant neoplastic growths, and each cancer of each organ exhibits numerous subsets. Despite this heterogeneity, malignant neoplasms have a uniform characteristic—the ability to invade host tissues and produce metastases. The most fearsome aspect of cancer is metastasis. This fear is well based. There have been great improvements in diagnosis, general patient care, surgical techniques, and local and systemic adjuvant therapies, but most deaths from cancer are still due to metastases that are resistant to conventional therapies. Continual empiricism is unlikely to produce significant improvement. Rather, a better understanding of the metastatic process and the development of biologic heterogeneity in neoplasms should provide a basis for new approaches to more effective therapy. Identification of specific properties unique to metastatic cells is an obvious prerequisite.

The process of cancer metastasis consists of linked sequential steps. Many investigators (1–3) but not all (4) view this process as favorable to the survival of subpopulations of metastatic cells pre-existent in the primary neoplasm (5). Metastasis begins with the invasion of the surrounding normal stroma either by single tumor cells with increased motility or by a group of cells from the primary tumor. Once the invading cells penetrate the vascular or lymphatic channels, they may grow there, or a single cell or clump(s) of cells may detach and be transported within the circulatory system. Tumor emboli must survive the host’s immune and nonimmune defenses and the turbulence of the circulation, arrest in the capillary bed of receptive organs, extravasate into the organ parenchyma, proliferate, and establish a micrometastasis. Growth of these small tumor lesions requires the development of a vascular supply and continuous evasion of host defense cells. When the metastases grow, they can shed tumor cells into the circulation and thus produce metastasis of metastases (1,2).

There is now wide acceptance that many malignant tumors contain heterogeneous subpopulations of cells with differences in such properties as growth rate, antigenic and immunogenic status, cell-surface receptors and products, response to cytotoxic agents, invasiveness, and metastatic potential. For production of clinically relevant metastases, all of the steps outlined in figure 1 must be completed. Failure to complete one or more steps of the process eliminates the cells. For this reason, the failure of most tumor cells to produce a metastasis can be due to different single or multiple deficiencies, such as inability to invade host stroma, a high degree of antigenicity, lack of aggregation, inability to arrest in the capillary bed, and above all, inability to grow in a distant organ’s parenchyma. Searching for a uniform factor that prevents tumor cells from producing metastasis is therefore unproductive.

During the last decade, numerous laboratories have succeeded in isolating cells with different metastatic properties from a variety of experimental rodent and human neoplasms. The availability of these selected subpopulations with known quantitative and/or qualitative differences in metastatic potential has facilitated numerous studies on the biochemical characterization of the “metastatic phenotype” (3,6–8). With the new advances in molecular biology, active research has been launched to identify (a) the genetic control of the metastatic phenotype and/or (b) the genes responsible for regulating discrete steps of the metastatic process.

It is unlikely that a complex process such as cancer metastasis is regulated by one gene; there is much evidence (3,9–12) to support the concept that this process is regulated by the activation and/or deactivation of many specific genes. Each discrete step of metastasis (e.g., invasion or extravasation) is probably regulated by transient or permanent changes at the DNA or RNA level in different genes. Moreover, the specific family of gene products may be different for each histologic tumor type. The use of clonal populations of tumor cells for analysis of genetic mechanisms of metastasis through comparative techniques is complicated by the fact that, over weeks or months of continuous culture, some clonal populations are unstable with regard to full metastatic competency (13, 14). Although this problem can be circumvented by use of polyclonal populations of metastatic cells (13), repeated in vivo assays to validate metastatic competency are mandatory.

Genetic control of the metastatic phenotype has been recently addressed by the use of several related approaches. Investigators have performed DNA-mediated transfer experiments in an attempt to isolate genes involved in metastasis, but to date, this approach has not identified any “candidate genes.” These experiments involve the transfer of the metastatic phenotype to tumorigenic, nonmetastatic cells by transfection of high-molecular-weight DNA. The feasibility of using this technique to identify genes that may regulate metastasis depends on two assumptions:

1. Transfection and assays to select genes with metastatic transfectants must be highly efficient.
2. Recipient tumorigenic, nonmetastatic cells must have a single dominant gene capable of switching on the entire metastatic process or a single gene that is complementary to an essential gene and that either is not expressed or is not present in cells already expressing all other genes controlling metastasis.
Transfection of a single gene has shown that some oncogene classes such as ras, src, myc, mos, raf, fes, fms, sis, and p53 can induce metastatic competency in some recipient cells. Although the mechanism of action of these various oncogenes is not clear, a multigene cascade presumably involving signal transduction pathways is implicated (9,10,15). Independent transfections with Ela, H-2k, or H-2D genes have produced a decrease in the metastatic competency of some highly metastatic cells (10,16). The effects of a transfected gene depend on the properties of the gene per se, the point of integration into the genome, the genetic background of the recipient cell, environmental influences, the stability of the transfection, and the host and assay systems used (9,11,15). Thus, a finding in one tumor system does not necessarily negate a finding in another tumor system. In any event, transfection of an oncogene(s) into appropriate cells may provide a system for studying biochemical properties of metastatic cells, since this procedure can produce a large number of variants (3,10).

Differential ("plus–minus") screening of cDNA libraries constructed from metastatic and nonmetastatic cells of the same tumor type has been used to identify candidate genes in metastasis. This technique relies on two premises:

1. that phenotypic changes associated with metastasis reflect changes in the synthesis and activity of many gene products and
2. that these products are regulated by transcriptional and posttranscriptional mechanisms manifesting themselves as quantitative changes in steady-state levels of numerous messenger RNA (mRNA) transcripts.

The method allows a search for genes that have either preferential or decreased expression in metastatic cells, compared with expression in nonmetastatic counterparts. This technique has led to the recent identification of mRNAs that are differentially expressed in metastatic and nonmetastatic cells. Examples include fibronectin (17), transin (18), murine calcium-binding protein (19), reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 5 (20), and human acidic ribosomal phosphoprotein P2 (21). Other mRNAs identified correlate with metastasis in murine melanoma (12,22) and murine large cell lymphoma (23). Unfortunately, the sensitivity of this technique is limited; it cannot detect transcripts present at a level below 0.01% in the mRNA population.

The more sensitive "subtractive hybridization" technique has been used to construct cDNA libraries from metastatic and nonmetastatic cells. This method involves physical removal of sequences common to both RNA populations under study and allows the isolation of mRNA transcripts constituting a percentage of the mRNA population as low as 0.001% (24). Hence, subtractive hybridization coupled with differential screening could increase the chance of cloning competent genes that are required, but are not of themselves sufficient, to significantly alter metastatic behavior (12).

In this issue of the Journal, Phillips and associates describe their use of these techniques in the isolation and partial characterization of one cDNA clone (pGM21) that is overexpressed in two different metastatic DMBA8 rat mammary adenocarcinoma model systems. Recently, other investigators using similar techniques isolated two other genes, WDNM1 (25) and WDNM2 (26), which have decreased expression in metastatic cells, compared with that in nonmetastatic cells in the same DMBA8 rat mammary adenocarcinoma system. Phillips and colleagues correctly point out that it is still unclear whether these gene(s) exert direct or indirect effects on metastasis. Nevertheless, there is much evidence (12,17–26) to support the concept that a complex process like cancer metastasis is regulated by the activation and/or deactivation of multiple specific genes.

Several biologic criteria must be fulfilled to determine whether an isolated (candidate) gene plays a direct role in regulating the metastatic phenotype. The gene of interest must be identified and its pattern of expression determined. Full-length cDNA clones should be isolated, incorporated into eukaryotic expression vectors, and transfected into the appropriate recipient cells. In addition, the phenotype should be assayed in a relevant model system: orthotopic implantation into anatomically correct organs (27). Finally, the reintroduced gene must be intact and functional.

Genes that are transcriptionally more active or less active in metastatic cells than their nonmetastatic counterparts could be tested in selected populations of cells with known differences in metastatic potential. These differences should be quantitative (i.e., high vs. low metastatic potential) and qualitative (i.e., deficiencies in a single step or multiple steps in the process of metastasis). The introduction of anti-sense strand RNA molecules that selectively inhibit the activity of genes and block the production of specific proteins could be used to confirm the role of a particular gene in regulation of one or more steps in the metastatic process.

The recent identification of novel genes that are associated with quantitative changes in the steady-state levels of mRNA in metastatic versus nonmetastatic cells adds important evidence to support the concept that cancer metastasis is not a random process; it is a highly regulated process that can now be studied on the molecular level. This new knowledge should eventually lead to the design and implementation of more effective therapy for this dreaded disease.
Evolution of tumor cell heterogeneity

References

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