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PROSPECT

Quantitative Variations in Gene Expression: Possible Role in Cellular Diversification and Tumor Progression

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Abstract Changes in the quantitative expression of certain genes or in the amounts of their products can quickly stimulate progression to the metastatic phenotype. This has been done experimentally by transferring dominantly acting oncogenes such as c-H-*ras*[EJ] into susceptible cells or more recently by interfering with metastasis suppressor genes. In vivo such rapid qualitative changes in dominantly acting oncogenes or suppressor genes occur only rarely, and progression to highly metastatic phenotypes is thought to occur through a process involving the slow stepwise progression of a subpopulation of neoplastic cells to more malignant states. Such slow changes can be reversible and need not involve known dominantly acting oncogenes or metastatic suppressor genes, consistent with clinical and experimental observations on naturally occuring, highly advanced metastatic tumors. An important element in the natural progression of tumors to more malignant states may be their ability to circumvent host environmental controls that regulate growth and cellular diversity. They also evolve into heterogeneous cellular phenotypes, a process that appears to mainly involve quantitative changes in gene expression but can be rapidly stimulated in cell culture by the introduction of a dominantly acting oncogene or inhibited by the introduction of a suppressor gene. The oncogenes and suppressor genes that affect malignancy may control important steps in the quantitative regulation of sets of genes that are ultimately responsible for the cellular alterations seen in adhesion receptors, cell motility responses, cell-cell communication components, degradative enzymes and their inhibitors, growth factor receptors, components that aid in escape from host surveillance mechanisms and others that are important in malignancy. Highly malignant cells that have slowly evolved in vivo may contain only a few qualitative gene changes but have undergone extensive cycles of diversification and accumulation of quantitative changes in the expression of genes that encode products that are related to malignancy and metastasis. Thus highly malignant cells can arise quickly due to specific qualitative changes in critical controlling genes or more slowly by less critical qualitative genetic changes together with cycles of cellular diversification and accumulation of quantitative changes in gene expression.

Key words: transformation, malignancy, metastasis, gene regulation, cancer

One of the most important but least understood aspects of cancer is the progression or change of tumor phenotype from benign to malignant and eventually to metastatic [1]. In its natural setting tumor progression occurs slowly due to the accumulation of relatively rare genetic changes [2–5], but these qualitative genetic changes may initiate events that eventually lead to more widespread changes in gene regulation that typify tumor progression [2,4]. Tumor progression to more malignant phenotypes is mainly characterized by quantitative changes in gene expression—in oncogenes, suppressor genes, differentiation genes, and genes associated with growth, invasion, and metastasis [3,4]. In terms of the number of changes, it is less characterized by qualitative (structural) alterations of genes (amplifications, mutations, deletions, translocations, and other genetic aberrations) than by quantitative changes in gene expression [4,6]. This Prospect will briefly discuss the important role that quantitative changes in gene expression plays during tumor progression.

As tumors progress, they are thought to be less responsive to host microenvironments and cellular controls that regulate growth and differentiation. Some malignant cells eventually gain autonomy from host tissue and systemic regulation—an important property of highly advanced tumors [7]. The host microenvironment is also important in regulating tumor progression, providing tumors with soluble and insoluble normal cell products that can modulate tumor cell

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properties and responses to host soluble, cellular, and extracellular matrix signals [4,8].

An important property of highly advanced tumors is their ability to rapidly diversify into heterogeneous phenotypes [4,6,9]. Although not strictly a property of tumor cells, the cellular heterogeneity seen in malignant tumor cell populations is usually more pronounced than in the cells of counterpart benign or normal tissues [4,5,9,10]. In normal tissues, soluble paracrine molecules as well as cellular and matrix interactions may combine to stabilize cellular phenotypes into less fluctuating states of diversity than seen in isolated single cells or in tumor cells evolving in the same tissue. Once removed from their normal microenvironments, however, normal cell populations show more diversity in their cellular properties [11]. Such differences in cellular diversity may be due to adoptive microenvironmental changes that individually affect each cell and result in quantitative differences in gene expression among individual cells. In malignant cell populations diversification can occur independent of (or at least less dependent of) the microenvironment, resulting in heterogeneous cellular phenotypes that cannot be regulated by host cells and tissues.

As tumor cells diversify they also undergo clonal selection in their host [3]. If host selection is effective at limiting the growth or eliminating a large fraction of cells within a tumor, this may result in surviving cells that display altered properties. An interesting result of this restriction of diversity of heterogeneous tumor cell populations is that subsequent diversification often occurs, resulting in even more heterogeneous cellular phenotypes [12]. Often a dominant subpopulation of tumor cells, however, eventually becomes the major tumor cell population due to advantages in growth and other properties [14]. Thus cycles of diversification and subsequent host selection of tumor cells occur until dominant malignant cell populations emerge that display highly autonomous phenotypes [2,6,12,13]. Thus progression probably results in waves of cellular diversification and restriction of diversity (clonal dominance) until malignant cell subpopulations acquire the correct properties to be highly successful in their host (Fig. 1) [2,6].

The rapid diversification of more autonomous cells within a tumor is not strictly characteristic of malignant or highly advanced tumor cells. In normal tissues some highly motile, invasive cells are capable of autonomous survival and growth at different sites. For example, certain embryonic cells, such as neural crest cells, primary gonocytes, and others, have the capacity of invasion and dissemination as single cells and can colonize distant sites from their origin. In adult normal tissues, moreover, wounding can initiate the events necessary for converting sessile, quiescent cells into motile, invasive cells capable of autonomous cellular division. Angiogenesis results in normally quiescent endothelial cells undergoing rapid change to motile, invasive cells that can proliferate in differing environments [15,16].

In contrast to the mainly quantitative, potentially reversible changes that probably occur during tumor cell diversification, qualitative and thus irreversible changes in DNA may fix certain changes in place during the progression of tumor cells, preventing reversion to previous states (Fig. 1). Although very important, qualitative events occur rarely and disparately among cells in a tumor and among different tumors of similar origin. If such qualitative changes in particular genes are critical to tumor progression, clonal dominance, and other characteristics of highly advanced tumors, then eventually all of the cells within a tumor should possess the qualitative change (Fig. 1).

ONCOGENES, TUMOR PROGRESSION, AND METASTASIS

The most common example of qualitative change in the genes of tumor cells is mutation in certain oncogenes and suppressor genes. In tumor cells oncogenes encode proteins that function abnormally, inappropriately, or at improper concentrations, resulting in the circumvention of the normal cellular controls that regulate cell division and the state of differentiation [18,19]. For example, in colorectal cancers the accumulation of multiple, different types of qualitative genetic change typifies the highly advanced malignant states [19]. Even in colorectal cancers, however, a range of genetic alterations has been found in each state, suggesting that other changes may also be important. Among the additional changes in colorectal cancer cells, quantitative differences in gene expression may determine, to some extent, the malignant properties of these cancer cells.

Single qualitative genetic changes, such as point mutations, deletions, amplifications, translocations, and others, occur in oncogenes and

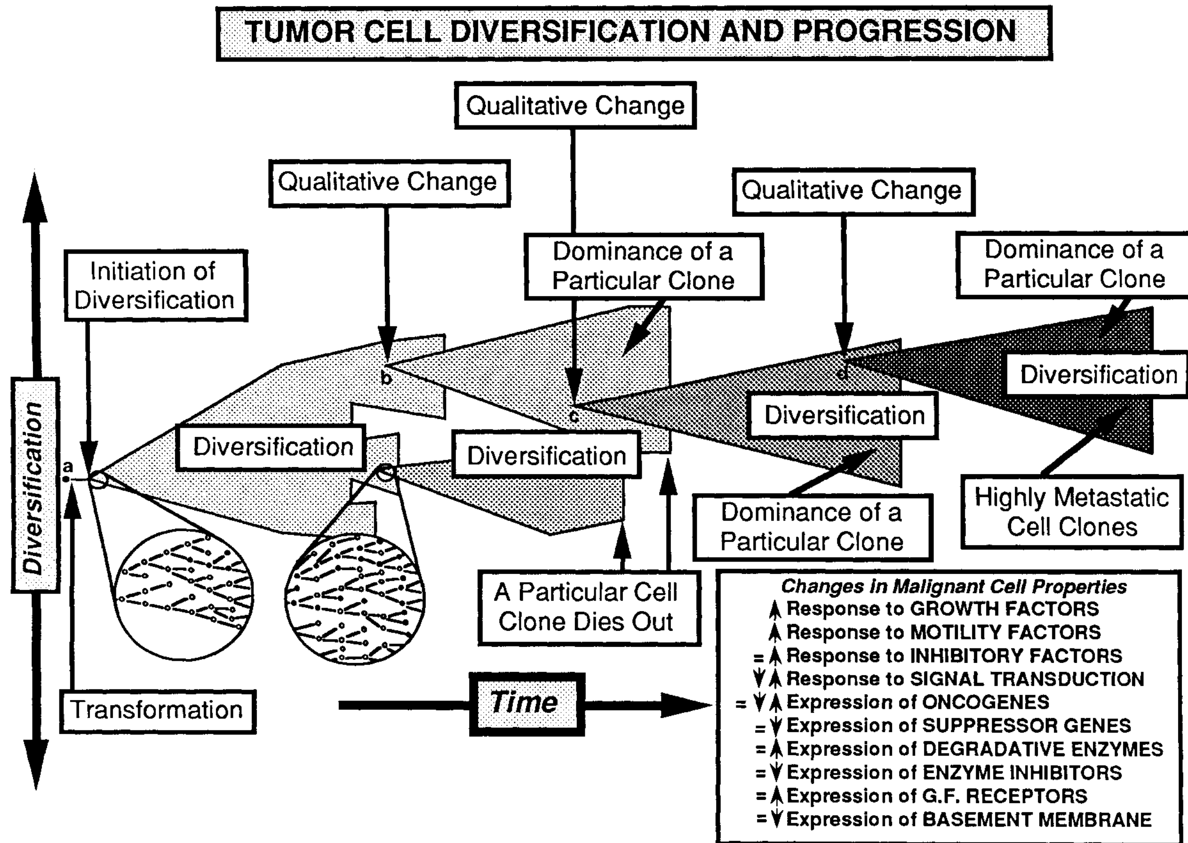

Fig. 1. Hypothetical example of how qualitative and quantitative changes might be related to tumor cell diversification and progression. **a:** A single cell is transformed, proliferates, and undergoes cellular diversification due to quantitative changes in gene expression. As the tumor cells diversify, particular cell clones begin to dominate the cell population due to growth advantages and host selection pressures. **b:** In one cell clone a qualitative change in a gene occurs that gives this clone an advantage over other clones in the population, and it proliferates and diversifies until clonal dominance again occurs. **c,d:** After several cycles of a qualitative genetic change, proliferation, and extensive quantitative changes in gene expression that drive diversification and eventually clonal dominance, the tumor cell population has progressed to a highly metastatic phenotype.

have been documented in chemically and spontaneously transformed cells. A more common feature of spontaneously transformed cells, however, is a change in oncogene expression or in the amounts of an oncogene-encoded product. Such single genetic events by themselves are unlikely to be the cause of cancer; further cellular changes are usually necessary [17–19]. This is best demonstrated in transgenic mice carrying activated oncogenes. Although the activated oncogenes are present in every cell, only a few cells are ultimately transformed and develop into tumors [20].

Since point mutations in oncogenes may or may not stimulate tumor progression, amplification of oncogenes has been proposed as an important mechanism for driving tumor progression [21]. Although amplification of oncogenes has been seen often in some clinical cancers, it is not a universal finding [22]. Amplification of oncogenes may be symptomatic, however, of additional, unrecognized changes in the cancer cell genome. The amplification of oncogenes and other genes could contribute to tumor progression without being the determinant [4–6].

Differences in the expression of oncogenes and in the concentrations of oncogene-encoded products have been proposed to be important determinants in tumor progression, especially to the metastatic phenotype [23,24]. Most of the studies that support this notion have utilized immortalized, aneuploid cells, and conversion to the metastatic phenotype occured rapidly upon transfection of several copies of dominantly acting oncogenes along with strong promoter/enhancer sequences [23–27]. Since the expres-

sion of oncogenes can differ between primary tumors and their metastases [22], differences in oncogene expression have been proposed to be an important event in the progression of tumor cells to the metastatic phenotype. Examination of a variety of primary and secondary tumors, however, revealed that oncogenes can be over-expressed, under-expressed, or expressed equivalently in metastases compared to primary tumors [4,22,28]. Thus, the qualitative changes seen in oncogenes or the quantitative changes in their expression may contribute to tumor progression, but they are unlikely to be the sole determinants [4,6]. Although metastases can show genetic alterations in oncogenes or over-expression of oncogenes or their encoded products, the data are not convincing in support of a strict causative role for oncogenes in the progression of naturally occurring tumors to metastases.

Progression to the metastatic phenotype occurs by successive change and evolution in vivo. It may also occur along different parallel pathways, some of which may be related to changes in oncogenes or their encoded products and some may not be. Under defined conditions the direct insertion of dominantly acting oncogenes into the DNA of a suitable recipient cell can result in rapid acquisition of the metastatic phenotype [23–27]. As mentioned above, this has been often accomplished using aneuploid, unstable, easily spontaneously transformable cells as recipients. In other experiments two dominantly acting or activated oncogenes were necessary, an event rarely seen in spontaneous tumors. Such rapid, dominantly acting qualitative changes that affect large numbers of cells in vitro are unlike the slow, sequential changes that characterize spontaneous transformation and tumor progression to the metastatic state in vivo [4,18]. The recipient cell type appears to be very important in oncogene transfection and gene insertion experiments. Some cells are highly resistant to oncogene-mediated conversion to the metastatic phenotype, and even within the same cell line there appears to be heterogeneity in the ability of dominantly acting activated oncogenes to cause metastatic conversion of individual cell lines or clones [6,28–30]. In some cases, the gene transfer techniques themselves may be as important as the actual oncogene in promoting metastatic conversion and can modify the expression of other unrelated genes [31]. Often multiple gene copies are transferred in gene transfer experiments, and the effects of their accompanying strong promoter/enhancer elements and locations in the genome are usually not considered. It is assumed that oncogene constructs are randomly incorporated into the genome, but just the opposite appears to be the case and nonrandom cytogenetic changes may occur concomitant with gene transfer [32,33]. Additional changes are probably necessary, and these changes are probably quantitative, reversible, and probably different in every cell receiving an oncogene construct. The quantitative expression of certain proteases and extracellular matrix components have been measured in oncogene transfected fibroblasts, and these gene products were expressed at different levels in different transfected cell subpopulations in vitro. With time in vivo, however, the expression of the degradative enzymes eventually reflected the metastatic phenotype of the cells [33], suggesting that host microenviromental factors may result in the emergence of dominant subpopulations of cells with particular malignant characteristics. Oncogene-mediated conversion of a cell to the metastatic phenotype is [23,27,32] or is not [29,30,35] highly correlated with the concentration of an oncogene-encoded product. Thus it seems fair to conclude that, in addition to oncogene insertion and expression, other cellular changes are necessary for progression to the metastatic phenotype.

METASTASIS SUPPRESSOR GENES AND TUMOR PROGRESSION

The existence of metastasis suppressor genes was originally predicted by cell fusion experiments where the metastatic properties of one cell could be suppressed by fusion with another cell type [36,37]. Several candidate metastasis suppressor genes have been identified by RNA subtractive hybridization techniques with cDNA libraries [38–42]. For example, Steeg et al. [42] used subtractive hybridization techniques to identify the *nm23* gene. This gene was found to be under-expressed in a variety of metastatic cell lines and in primary infiltrating breast ductal carcinomas of high metastatic potential. Affinity purified antibodies against the surmised *nm23* N-terminal peptide were used to immunoprecipitate a cytoplasmic and nuclear protein of the predicted size for the *nm23* gene, and it was found to be expressed in low quantities in highly metastatic cells [43]. The predicted *nm23* protein sequence was subsequently found to have an extraordinarily high identity with the prod-

uct of the *Drosophila* developmental gene *awd* [44]. Null mutations in the *awd* gene result in abnormal embyronic development and death. Recently the *awd* gene was shown to have a high degree of homology with the gene encoding a nucleotide diphosphate kinase [45], suggesting that the *nm23* gene product may function in microtubule assembly/disassembly or in signal transduction by regulating G proteins [46]. The regulation of microtubules could be important in mitotic spindle formation and cell motility, suggesting that aberrant mitotic events could result from changes in *nm23* structure or expression. The function in G protein regulation of second messenger pathways suggests that an altered *nm23* gene or its expression could result in altered regulation of a variety of genes [46].

Practically any gene that encodes a protein product that can inhibit the metastatic process can be considered a metastasis suppressor gene. This includes genes encoding natural protease inhibitors that block invasion or substances that inhibit tumor cell motility. Examples of the former are the tissue inhibitors of metalloproteinases (TIMPs) and plasminogen activator inhibitors (PAIs) [38]. Transfection of antisense TIMP RNA inhibited TIMP-1 expression and enhanced the malignant properties of mouse 3T3 cells, and administration of recombinate TIMP-1 inhibited in vitro invasion and lung colonization of mouse melanoma cells [47].

GENE EXPRESSION, DIVERSIFICATION, AND METASTASIS

Changes in gene expression can have profound effects on a cell's properties. Alterations in gene expression may alter cellular metastatic properties by increasing the amounts of degradative enzymes or decreasing their inhibitors, increasing or decreasing cell adhesion components, increasing growth factor receptors or modifying their signals, and altering cell-cell communication components, cell motility components, or components that allow a malignant cell to escape host surveillance mechanisms (Fig. 1). In some cases differentially expressed genes have been related to malignant properties. Mouse lymphoma variants over-expressing the mitochondrial gene *ND5* were shown to be more metastatic to the liver [40]. The *ND5* gene encodes the NADH: dehydrogenase in complex I of the electron transport chain, and over-expression of the *ND5* gene may allow highly metastatic lymphoma cells to escape macrophage-released cytostatic factors that act as respiration inhibiting molecules at the level of mitochondrial complex I [40]. Although some of the differentially expressed genes in metastatic cells have been identified, most of the differentially expressed genes and their products that are associated with metastasis (or lack of metastasis) are without a known function [48]. For example, highly metastatic mouse cells appear to over-express the *mts1* gene, which has a high homology with calcium-binding proteins but is of unknown cellular function [48].

Unfortunately, most of the experiments linking specific genes with the metastatic or nonmetastatic phenotype are correlative and do not focus on function. Future experiments will undoubtedly involve altering regulation of candidate genes and relating their expression more directly to metastatic properties. As an example of this approach, Kushtai et al. [39] transfected the c-*fos* oncogene into highly metastatic murine 3LL cells. Cell clones expressing high levels of the transfected c-*fos* gene also expressed elevated levels of H-2K and H-2D mRNA, synthesized high levels of cell surface H-2K^b, and had lowered metastatic capabilities. The explanation for these results is that the 3LL cells expressing high levels of cell surface H-2K^b were probably more immunogenic and rejected more readily by host response systems [50]. Although other explanations for these results are possible (see comments above on transfection techniques), the most obvious conclusion is that the c-*fos* oncogene is somehow regulating H-2 gene expression and synthesis of the H-2 histocompatibility antigens in 3LL cells. It undoubtedly has other effects as well.

Several, not just a few, genes are probably involved in the expression of the metastatic phenotype of a cell. Single gene transfer experiments will, therefore, not always be useful, unless the transferred genes are regulatory genes that affect many other genes that are involved in metastasis. Examples of possible regulatory genes are those genes that encode transcription regulatory factors, signal transduction components, and other gene products that can regulate several other genes [46].

As mentioned above, highly malignant cells exhibit rapid rates of phenotypic diversification, and this may be due to quantitative differences in the expression of several but not an unlimited set of genes. Ultimately this can result in a range of different immunological, biochemical,

enzymological, structural, and other cellular phenotypes in a tumor cell population [4]. In vitro transfection experiments with dominantly acting oncogenes suggest that rapid cellular diversification occurs concomitant with malignancy [6,30]. Qualitative events, such as alteration in a dominantly acting oncogene, would be expected to occur at a very low rate in vivo, but the tissue culture experiment described above does demonstrate that a possible relationship may exist between qualitative genetic changes, cellular diversification and metastatic potential. Stimulation of cellular diversification could thus be an important step in converting cells to more malignant phenotypes.

The mechanisms that control cellular diversification and genomic stability are obscure. The genes that control cellular stability may be related to cellular division and synthesis of DNA [5]. These genes or their products may be altered during tumor progression, resulting in extensive tumor cell diversity, host selection, and eventually acquisition of malignant and metastatic properties. Volpe [5] suggests that these genes should be called stability genes, and they include the genes involved in karyokinesis and the repair, recombination, and replication of DNA. In addition to inherent (genetic, qualitative) defects in cellular replication machinery that could result in loss of cell stability and diversification, epigenetic factors, such as tissue and stromal molecules, can also control cellular diversity and gene expression programs [4,51]. Since even removal of normal cells from their usual microenvironments can result in loss of tissue-specific gene regulation and control [11], changing microenvironments could be very important in determining states of diversification and gene expression. In normal tissues as well as tumors individual cells experience variations in the concentrations of nutrients, oxygen, growth and differentiation factors and inhibitors, hormones, enzymes, ions, and other regulatory factors. Thus normal cells may be more stable than tumor cells in the face of changes in their microenvironments. Under certain microenvironmental conditions, however, even malignant cells can be forced to differentiate to essentially normal cells. By inserting teratocarcinoma cells into normal blastocysts, the malignancy of the implanted teratocarcinoma cells can be regulated by the microenvironment of the blastocysts. Not all malignant cells, however, develop into normal adult cells upon implantation into the blastocyst [52], suggesting that tumor cells can progress to a point where they are no longer affected by microenvironmental signals.

Tumor cellular diversity is not likely to be an event associated only with the transformed or malignant state of a cell. Normal cells also undergo diversification, especially during development. Even in adult organisms some cellular diversification systems are active, such as those involved in the diversification of lymphocytes in response to specific antigens. In this normal adult cell example, hypermutable and stable regions of gene families are rapidly rearranged into new genes that encode unique new molecules [53]. Many if not all of the gene products important in malignancy and metastasis are probably also normal gene products that are inappropriately and heterogeneously expressed by malignant cells [4]. Although we are just beginning to learn the identities of these genes and their encoded products, they are very likely to also be important in normal homeostasis and development [4,6,46].

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