Mechanisms of Tumor Progression

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Tumors often become more aggressive in their behavior and more "malignant" in their characteristics during their life history, although the time course may be quite variable. This phenomenon has been termed tumor progression, and Foulds (1, 2) first pointed out that the process appears to develop in a stepwise fashion through qualitatively different stages. Others, including the author, have subsequently suggested that this biological and clinical progression might reflect, at least in part, the sequential appearance within the tumor of increasingly genetically altered subpopulations with new characteristics (3-5). It is the purpose of this brief discussion to summarize some current thoughts concerning tumor progression and suggest where present investigations may be leading.

Clinical and Biological Characteristics of Progression

The alterations in cellular behavior and morphology that accompany tumor progression have been well described (2, 6). The acquisition by the neoplastic cells of the capacity to invade locally and to metastasize remains the aspect of progression that is of greatest clinical significance; this is still the fundamental definition of malignancy. In recent years, many workers have begun to extend studies of the biological characteristics of metastasis to the specific molecules and metabolic pathways important in the successful invasion of adjacent tissue and colonization of distant sites (6, 7). Attempts are being made to define specific properties of metastatic cells and of the host environment in which they thrive. Primarily in experimental tumors, the relative importance of various factors, including proteolytic enzymes, plasminogen activators, tumor angiogenic factor, platelet agglutinating capacity, and membrane molecules such as lamin, fibronectin, and major histocompatibility complex gene products are all being actively explored (6, 7). As the biological role of each of these entities is being clarified, some workers are also beginning to extend these investigations on invasion and metastasis to human systems and to the molecular genetic level.

With respect to other aspects of tumor progression, there is also a tendency, with time, for tumor cell populations to increase their growth rate and to show further evidence of escape from local growth control mechanisms. Usually, this appears not to reflect a shortening of the cell cycle time, but rather an increase in the "growth fraction," the proportion of cells within the neoplastic population that continues to proliferate actively instead of progressing to terminal differentiation or cell death (8). In some circumstances, this further escape from growth regulation may be related to altered response to circulating hormones, through loss of specific receptors or other mechanisms (9).

It is also common for tumors, as they become more malignant, to show morphological and metabolic alterations that are generally interpreted as loss of differentiation. Organelles and metabolic functions necessary for specialized activities in the cell often decrease or disappear. There continues to be considerable debate as to whether this phenomenon reflects an actual "block" in differentiation or, again, simply an increased growth fraction, with a higher proportion of cells remaining in an "undifferentiated" proliferating state. Evidence is beginning to develop that there are certain limited "windows of differentiation" in which particular genes important in neoplastic development ("oncogenes") may function in certain cell lineages and stages of ontogeny (8, 10); thus an explanation of the level of differentiation within particular neoplasms may be approachable in terms of specific genes and gene products.

Tumor progression may also be accompanied by the elaboration of increased amounts of particular proteins by the malignant cells. Some may contribute to the capacity for invasion and metastasis (6, 7). Others, such as "inappropriate" hormones, may have significant clinical effects at distant sites (11). Decreased antigenicity and acquisition of drug resistance may also appear within the advanced neoplastic cell population, and these characteristics have obvious selective advantages for the tumor.

It is important to reiterate that there may be considerable variability in the time period and sequence in which these various aspects of tumor progression become apparent. In some instances, the properties of far advanced malignancy may be established before the neoplasm reaches macroscopic size; in other cases, well differentiated slow growing tumors may persist for years before undergoing a relatively abrupt shift to more aggressive behavior. It is also common, particularly with rapidly growing cancers, to find heterogeneity within the tumor regardless of which parameter is measured, so that some components of the neoplastic population may appear much further advanced than others in the degree of tumor progression.

Clonal Evolution in Neoplastic Populations

Considerable biochemical, cytogenetic, molecular genetic, and immunological evidence now indicates that most neoplasms arise from a single altered cell, with the progeny of that cell expanding as a neoplastic "clone" (4, 12, 13). As noted above, a number of workers have suggested that the clinical and biological events of "tumor progression" represent the result of sequential selection of variant subpopulations within this clone. It has also been hypothesized that such "clonal evolution" might result from enhanced genetic instability within the tumor cell population, which increases the probability of further genetic alterations and their subsequent selection (3-5, 14). In this concept of clonal evolution, most variants that arise in the tumor cell population do not survive; but those few mutants that have an additional selective growth advantage expand to become predominant subpopulations within the neoplasm and demonstrate the characteristics that we recognize as tumor progression. The continued presence of multiple subpopulations within the tumor provides the basis for the heterogeneity found in advanced neoplasms.

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that is also typically observed.

Much of the evidence supporting this clonal evolution concept has been derived from chromosome studies, where somatic genetic changes can be readily visualized. In general, advanced cancers show more extensive chromosomal aberrations than do early stages of neoplasia (15–17). Because it has been difficult to do serial investigations on the common human cancers, however, the most significant sequential data have been obtained from repeated studies on individual cases of leukemia and lymphoma. Best documented are the findings in chronic granulocytic leukemia, in which the cells in the early indolent stage of the disorder typically show only the t(9;22) chromosome translocation involving the c-abl oncogene that results in the Philadelphia chromosome, but the terminal accelerated phase of the disease apparently represents overgrowth of this leukemic population by one or more subclones having additional karyotypic changes (17, 18). Similar associations have been demonstrated in the progression of myeloid preleukemia to frank leukemia and in the cells of acute leukemia in relapse (16). Interestingly, the clinical progression observed in chronic lymphocytic disorders appears chiefly to involve alterations in host response, but occasionally a very aggressive form of tumor progression, such as Richter’s syndrome, has been associated with the appearance of a newly expanded subpopulation of the original lymphocytic clone with marked additional genetic alterations demonstrable in the karyotype (19).

There are also a few chromosomal reports on human non-hematopoietic tumors and on experimental animal tumors in which it has been possible, through serial biopsies or through the study of a primary tumor and its metastases, to relate more aggressive tumor growth to additional genetic changes in the neoplastic cells (20–22). In most human cancers, however, it has been necessary to infer a sequence of events by examination of tumors in different patients at different stages of development. In several cases, these findings have begun to suggest specific genes that might be involved in the evolutionary process. Thus, in neuroblastoma there are recent data demonstrating additional chromosome changes associated with more aggressive stages of disease and, perhaps more importantly, showing that these additional changes may include appearance in the metaphases of a homogeneously staining region or double minutes that represent gene amplification units involving the oncogene N-myc (23).

A similar relationship between specific somatic genetic changes and more advanced stages of neoplasia may be represented by our recent findings in melanoma. We have observed that an extra dosage of chromosome 7, and specifically the short arm of this chromosome (7p), that is present in many advanced melanomas appears to be associated with expression on the tumor cells of the receptor for the epidermal growth factor (24). Since the human protooncogene c-erbB appears to code for a portion of the epidermal growth factor receptor, this may represent cytogenetic evidence of a significant alteration in oncogene function contributing to further selective growth advantage in already malignant cells.

In other collaborative studies, with Dr. Carlo Croce and his colleagues, we have utilized combined cytogenetic and molecular genetic studies of an acute lymphocytic leukemia cell line to suggest how sequential activation of two oncogenes might explain the aggressive clinical behavior of the neoplasm (25). These cells had both the t(8;14) translocation characteristic of Burkitt’s lymphoma and a t(14;18) translocation commonly associated with relatively indolent follicular lymphoma. We hypothesized that the latter translocation, which involves the putative oncogene bcl-2, occurred first and that the subsequent acquisition of the t(8;14) rearrangement, which results in deregulation of the c-myc oncogene, converted a low-grade lymphocytic tumor into a rapidly expanding neoplasm presenting as acute leukemia (10, 25).

These preliminary findings and speculations indicate how modern techniques may permit the clonal evolution hypothesis of tumor progression to be explored at the level of specific genes and gene products. Before commenting on both the opportunities and the difficulties of further work in this direction, however, I will discuss briefly the mechanisms that may underlie genetic instability and clonal evolution in tumor cell populations.

Mechanisms of Clonal Evolution

Genetic Instability of Tumor Cells. There is evidence that most neoplastic cells are more genetically unstable than comparable normal cells and that this may be a major factor contributing to the phenomenon of clonal evolution (26). The supporting data come from both direct observation and experimental studies. When tumors are examined histologically, one is often struck by the presence of obvious mitotic abnormalities. In fact, such findings led to the early theories of Boveri (27) and von Hansemann (28) concerning an important role of chromosomal alterations in the development of cancer. Direct histologic studies also suggest that genetic instability represented by mitotic abnormalities may become more pronounced as a neoplasm evolves. In advanced cancers, a wide range of mitotic variants is commonly observed with each cell generation, as compared to relatively few in early benign lesions (29).

Experimental data indicating that tumor cell populations are more genetically labile than comparable normal cells are being reported with increased frequency. Both in vivo and in vitro, there is evidence that neoplastic cells may be more susceptible than comparable normal cells to chromosome breakage, nondisjunction and ploidy changes, sister chromatid exchange, and other genetic alterations (14, 26, 30, 31). There are even limited experimental data indicating that this enhanced mutability increases with tumor progression (26, 31). Ling et al. (31) have pointed out that high, and changing, rates of mutation play an important role in what they call the “dynamic heterogeneity” of malignant cell populations with respect to such properties as drug resistance and capability for metastasis.

In considering the basis for such phenomena, it is assumed that for the vast majority of cancer patients there is no inborn abnormality in genetic stability and that increased lability within the neoplastic clone is therefore the result of an acquired alteration. Many kinds of acquired defects have been suggested by various workers, and some supporting evidence has been developed in studies of different tumors.

Single gene mutations of various types could destabilize the genome. A mutation might result in a defect in DNA repair similar to those in the constitutional “chromosomal fragility syndromes.” An acquired abnormality in a DNA synthetic enzyme, increasing the utilization of more error prone pathways, could have the same result, as could an acquired defect in the proteins of the mitotic spindle, resulting in abnormalities of the mitotic process itself. There are reports consistent with the action of each of these suggested types of mutations in different neoplastic cell populations (32–34). In addition to such single gene lesions, one can also postulate that chromosomal alterations, once established within the tumor cell population, may themselves contribute to the contin-
Continuing destabilizing effect on the host cell genome (14).

Effects may be present in other individuals and families. There are increased probability of nondisjunction (26, 34, 35). Other types of cytogenetic abnormalities could have similar effects.

Although certainly not inclusive, these considerations indicate that various kinds of acquired specific mutations and chromosomal alterations may underlie the observed genetic lability in different tumor cell populations. There is currently little firm evidence to suggest the relative importance of different types of lesions, although Cairns (34) has suggested that gross chromosomal abnormalities may be of greater importance in human neoplasia than point mutations. It should also be recognized that various types of genetic change could occur at different times during tumor development, even within the same neoplasm. As noted above, chromosomal alterations in a tumor might result from a destabilizing gene mutation acquired earlier and then in turn contribute to a cascade of increasing instability. There is also evidence that the degree of cellular heterogeneity within the neoplastic clone may itself contribute to the level of genetic instability, through unexplained interactions among the coexisting subpopulations of tumor cells (36).

In addition to these considerations, there is also a small segment of the human population, and particularly in the pediatric age group, in which increased genetic instability in neoplastic cells may not result from an acquired alteration but rather reflect an inherited gene defect present in all cells of the body. Individuals with the chromosomal fragility syndromes (Bloom's syndrome, Fanconi's anemia, ataxia telangiectasia, xeroderma pigmentosum, etc.) (26, 37) apparently have an inherited defect in DNA repair or in some other aspect of DNA "housekeeping," although the details are not completely understood in all instances. As a somewhat oversimplified generalization, it can be suggested that genetic lability in all of the patient's cells, resulting from the inherited gene defect, may lead to random chromosome aberrations, cytogenetically abnormal clones with a high potential for evolution, and ultimately the increased incidence of neoplasia that characterizes these disorders (26).

It is also possible that inherited gene defects with similar effects may be present in other individuals and families. There have been various families described, for instance, with increased frequency of blood disorders, bowel cancer, or melanoma in which there is at least preliminary evidence suggesting an inherited defect in karyotypic stability (38, 39), and this may also be the case in some kindreds that show a general familial tendency to increased frequency of blood disorders, bowel cancer, or melanoma in which there is at least preliminary evidence suggesting an inherited defect in karyotypic stability (38, 39), and this may also be the case in some kindreds that show a general familial tendency.

Finally, it is worth noting that in addition to these inherited and acquired mechanisms that may produce genetic instability in different neoplasms, it is also possible that extracellular factors may contribute to the sequential genetic alterations involved in clonal evolution. If, for instance, an oncogenic virus is involved, its incorporation into the genome of the cell may not only trigger the initial transformation event but also have a continuing destabilizing effect on the host cell genome (14). Similarly, the continued presence of a long lived carcinogenic chemical or radioisotope in an individual, or repeated doses of such clastogenic materials through occupational or other exposure, could also result in sequential mutations within the tumor. Even the mutagenic therapeutic agents used in cancer treatment (radiation, chemotherapy) may contribute significantly in this way to the later stages of clonal evolution and tumor progression in some patients. If we are to understand better the clinical and biological phenomenon of progression, considerably more research should be done on the various factors involved in the apparent genetic instability of neoplastic cells.

Host Factors in Clonal Evolution. In addition to the alterations within the neoplastic cells that play a role in tumor progression, one must also consider those factors in the host environment that provide the selective pressures determining which mutant cells expand into predominant subpopulations at various times. Despite continuing debate on the efficacy, and even the existence, of "immune surveillance," it seems clear that the host immune system represents one type of selective pressure on evolving neoplasms, particularly in the early stages (40). Immunologically nonspecific cytotoxic mechanisms directed against tumors by such cells as the macrophage and natural killer cells, as well as soluble mediators like interferon and tumor necrotizing factor, may also be of major significance (8, 40, 41).

In addition to aspects of immune regulation, there also remains much to be learned concerning other substances that may influence growth at the local level (e.g., growth factors, chalones) and to which the neoplastic population may respond abnormally (8). Although several human "protooncogenes" have recently been related to growth factors or their receptors (8, 40, 42, 43) and specific "tumor growth factors" have been tentatively identified (8, 44), our understanding of normal and abnormal growth regulation at the local level remains remarkably limited. Much more needs to be learned concerning the tissue specific stimulators and inhibitors of cellular proliferation that act over short distances and brief time periods to maintain normal structure and repair injury. Without a better understanding of the sequence of extracellular and intracellular events in normal tissues, it is very difficult to define critical alterations in neoplastic cells.

One important aspect may be damage to the microenvironment of an early neoplasm, leading to serious disruption of local regulatory mechanisms and thus playing a significant role in subsequent tumor progression. In considering chemically induced cancer in the rat liver, Farber and Cameron (45) have stressed the cytotoxic effects of many of the carcinogenic agents used, as well as the selective advantage to emerging tumor cells of resistance to this cytotoxicity. Similar considerations may be important in the pathogenesis of certain human neoplasms. For example, the leukemogenic effects in humans of such chemicals as benzene and the alkylating agents used in cancer therapy may reflect not only their direct mutagenic influence on marrow cells but also their damage to the local microenvironment, which allows potentially leukemic clones to expand (46). Any circumstance that alters the local environment and allows competition among subpopulations of a developing tumor may have a significant influence on the continuing evolution of the neoplastic population.

Other host factors may also be important in both the early and later stages of human neoplasia. The environmental pressures generated by the general health and nutrition of the patient, his exposure to infectious agents, and even the therapeutic efforts of the physician may all serve to accelerate the appearance of new sublines within the tumor (2, 4). In the areas of Africa where Burkitt's lymphoma is endemic, chronic infection with malaria and with the Epstein-Barr virus produces both damage to the immune system and continuing stimulation of lymphoid cells, apparently increasing the opportunity for a
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A variety of mechanisms by which oncogenes can be “activated” have also been described. These may involve such diverse processes as point mutations in the gene, amplification or deletion of the gene, or chromosomal translocation, with or without structural change in the gene (10, 23, 40, 42). In all cases the products of oncogenes have been identified, as nucleoproteins, tyrosine kinases, growth factors, or their receptors (8, 40, 42, 43). Data have also been developed, for a few of these genes, on the specific cell types and stages of cellular differentiation in which they are functional, suggesting a specific role for certain oncogenes in particular tumors (10).

A final consideration concerns the ultimate significance of these concepts and investigations of tumor progression with respect to the diagnosis and treatment of human cancer. Even with our limited understanding of the specific molecular events involved in clonal evolution, it is now widely accepted that most of the common human cancers (e.g., breast, lung, bowel), at the time of clinical presentation, have undergone extensive and variable somatic genetic changes and selection within the individual host and that the process is a continuing one. This has helped to explain the difficulty in finding consistent alterations in the common cancers that would permit specific therapy to be designed. It has also offered little hope of reversing, through differentiating agents, the aggressive growth patterns of far advanced neoplastic cells.

In other geographical areas, human T-cell leukemia virus I may have a similar role in T-cell neoplasia, stimulating continued T-cell proliferation and thus increasing the probability that a chromosomally abnormal clone will develop (47). The same can be said for specific aspects of tumor progression as they are seen in different patients. As with all models, the limitations of long established tumor cell lines both in vivo and in vitro must be recognized, and particularly so when the questions being asked concern the stepwise acquisition of neoplastic properties in the natural host.

An additional difficulty is the problem of appropriate models. There are very few human tumors, except in the hematopoietic system, where it is possible to obtain serial specimens in order to follow the development from benign to malignant, or from less aggressive to more aggressive, over a long period of time. Certain lesions presenting on the skin, and particularly the nevus-melanoma sequence, are beginning to be utilized for such studies (22), but most of the initial investigations must still be done in experimental animals and in tissue culture. The hope is that as molecular details are worked out in such models, it will be possible to carry them back to the various stages of neoplasia as they are seen in different patients. As with all models, the limitations of long established tumor cell lines both in vivo and in vitro must be recognized, and particularly so when the questions being asked concern the stepwise acquisition of neoplastic properties in the natural host.

Future Directions

The most exciting development in fundamental cancer research in recent years has been the recognition and investigation of individual genes important in tumor development (40, 42, 43). These were first identified in mammalian cells through homology with retroviral oncogenes and through transfection assays. Additional putative human oncogenes are now being characterized through their involvement in nonrandom chromosome alterations in tumor cells (10, 40, 42). In some cases the products of oncogenes have been identified, as nucleoproteins, tyrosine kinases, growth factors, or their receptors (8, 40, 42, 43). Data have also been developed, for a few of these genes, on the specific cell types and stages of cellular differentiation in which they are functional, suggesting a specific role for certain oncogenes in particular tumors (10).

The current identification of particular genes associated with various characteristics of human malignant growth opens the possibility of finding altered gene products that might be susceptible to specific immunological or chemotherapeutic attack, at least in those tumors where there is a structural change in the oncogene (10, 42). Certain encouraging results with “differentiating agents” such as the retinoic acid derivatives have also recently indicated, in both humans and model systems, that even aneuploid cell populations can in some circumstances be restored to a normal balance between proliferation and differentiation (53). Additional biological agents with antitumor activity are being identified. There are also very recent studies demonstrating that DNA methylation patterns may play an important regulatory role in gene function and that agents such as 5-azacytidine, which alter these patterns, may be able to reverse certain characteristics of “progression” in neoplastic cells (54). The outlook is now certainly more hopeful than when some of these views on clonal evolution were first expressed a decade ago (4). We must continue to acknowledge, however, the validity of the concluding statement by Foulds, in a guest editorial on tumor progression in these pages in 1957: “It is possible, as long suspected by some, that the characters that most decisively govern the outcome of neoplasia and its response to treatment are as yet unknown” (1). Detailed study of
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the molecular genetic basis of tumor progression during the next few years should help to delineate some of those "characters."

References

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