Disorders in cell circuitry during multistage carcinogenesis: the role of homeostasis

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The multistage process of carcinogenesis involves the progressive acquisition of mutations, and epigenetic abnormalities in the expression, of multiple genes that have highly diverse functions. An important group of these genes are involved in cell cycle control. Thus, cyclin D1 is frequently overexpressed in a variety of human cancers. Cyclin D1 plays a critical role in carcinogenesis because (i) overexpression enhances cell transformation and tumorigenesis, and enhances the amplification of other genes, and (ii) an antisense cyclin D1 cDNA reverts the malignant phenotype of carcinoma cells. Therefore, cyclin D1 may be a useful biomarker in molecular epidemiology studies, and inhibitors of its function may be useful in both cancer chemoprevention and therapy. We discovered a paradoxical increase in the cell cycle inhibitors protein p27Kip1 in a subset of human cancers, and obtained evidence for homeostatic feedback loops between cyclins D1 or E and p27Kip1. Furthermore, derivatives of HT29 colon cancer cells with increased levels of p27Kip1 showed increased sensitivity to induction of differentiation. This may explain why decreased p27Kip1 in a subset of human cancers is associated with a high grade (poorly differentiated) histology and poor prognosis. Agents that increase cellular levels of p27Kip1 may, therefore, also be useful in cancer therapy. Using an antisense Rb oligonucleotide we obtained evidence that the paradoxical increase in pRb often seen in human colon cancers protects these cells from growth inhibition and apoptosis. On the basis of these, and other findings, we hypothesize that homeostatic feedback mechanisms play a critical role in multistage carcinogenesis. Furthermore, because of their bizarre circuitry, cancer cells suffer from ‘gene addiction’ and ‘gene hypersensitivity’ disorders that might be exploited in both cancer prevention and chemotherapy.

The current paradigm of multistage carcinogenesis

A variety of experimental and clinical studies carried out during the past century established the principle that cancers develop through a multistage process which can encompass an appreciable fraction of the lifespan of the species (1–3). Within the past few decades astounding progress has been made in our understanding of the cellular, biochemical and molecular genetic events that occur during this multistage process. A current paradigm is that this stepwise process reflects the progressive acquisition of activating mutations in dominant acting growth enhancing genes (oncogenes) and inactivating recessive mutations in growth inhibitory genes (tumor suppressor genes) (4). It is also apparent that epigenetic abnormalities in the expression of these genes also play an important role in carcinogenesis (1–3).

Following the initial discovery of oncogenes, ~20 years ago, it appeared that there might be a small number of such genes. However, since then over 100 oncogenes and at least 12 tumor suppressor genes have been identified, and the list keeps growing (5–7). Moreover, a single colon cancer cell frequently contains defined mutations in multiple genes (four or more) plus numerous less well defined mutant and/or aberrantly expressed genes, as well as gross chromosomal abnormalities. Indeed, in human colon tumors ~25% of all loci show loss of heterozygosity, and in cancer cells with defects in DNA mismatch repair thousands of loci can be mutated in a single cancer cell (6,8,9). Presumably, these widespread changes reflect the deleterious effects of mutagens, both exogenous and endogenous, as well as various types of genomic instability acquired during tumor development. In the subset of familial cancers some of these mutations, usually in tumor suppressor genes, are inherited, thus enhancing the multistage process of carcinogenesis.

Because of the large number and diverse functions of the known oncogenes and tumor suppressor genes we have developed a classification scheme which is based on their specific biochemical functions (Table I) (10). The genes are divided into two broad functional categories, those that control intracellular regulatory circuitry and those that influence cell surface and extracellular functions. The first category is further divided into four subcategories: (1) genes that play a role in the responses of cells to external growth stimuli (i.e. genes that encode growth factors, cellular receptors, coupling proteins, and protein kinases that transduce information across the cytoplasm to the nucleus) and nuclear transcription factors that then increase or repress the expression of specific genes; (2) genes involved in DNA replication and repair; (3) genes involved in cell cycle control, including checkpoint functions; and (4) genes that determine cell fate, i.e. cellular differentiation, senescence and programmed cell death (apoptosis). Many of the oncogenes, for example ras, are in subcategory 1. Subcategory 2 includes the DNA excision and mismatch repair genes. Subcategory 3 includes the tumor suppressor genes Rb and p53. Recent studies on cyclins and cyclin-related genes and their abnormalities in cancer have rapidly expanded this subcategory (11,12). This subject is discussed in greater detail below. Subcategory 4 includes the bcl-2 family of proteins that regulate apoptosis. This category is of considerable importance since it is now apparent that the increased proliferation of cancer cells reflects a disturbance in the balance between de novo cell replication and terminal

Abbreviations: CDI, CDK inhibitor protein; CDK, cyclin-dependent kinase.
Abnormalities in cell cycle control proteins in cancer

Subcategory 3 in Table I represents a recent set of oncogenes and tumor suppressor genes, discovered as a result of the recent elucidation of the specific proteins that normally regulate the cell cycle in a variety of eukaryotic cells. As shown in Figure 1, the orderly progression of dividing mammalian cells through the G1, S, G2 and M phases of the cell cycle is governed by a series of proteins called cyclins, which exert their effects by binding to and activating a series of specific cyclin-dependent kinases (CDKs). This process is further modulated by the phosphorylation and dephosphorylation of CDK proteins by specific protein kinases and phosphatases and by a series of specific CDK inhibitor proteins (CDIs), including p16INK4a, p21Waf1 and p27Kip1 (Figure 1B) (11,12).

In recent years it has become apparent that carcinogenesis is frequently associated with mutations or abnormalities in the expression of various cyclins, CDKs and CDIs, in several types of human cancers (for review see refs 11,12). Thus, the cyclin D1 gene, which acts at the mid-portion of the G1–S transition, is often overexpressed in human breast, colon and squamous carcinomas, and several other types of cancer, and the cyclin E gene, which acts in late G1 is also overexpressed and dysregulated in a variety of human cancers (11,12). Indeed, increased expression of cyclin D1 is one of the most frequent abnormalities in human cancer since it occurs in ~60% of breast cancers, 40% of colorectal cancers, 40% of squamous carcinomas of the head and neck and 20% of prostate cancers (10–13). Furthermore, increased expression of cyclin D1 can be an early event in carcinogenesis, since it is also seen in precursor lesions of the colon, esophageal and breast (14,15 and unpublished data). It may, therefore, be a useful biomarker in molecular epidemiology and chemoprevention studies. It is of interest that the APC/β-catenin pathway regulates the expression of cyclin D1 (16,17), which may explain why cyclin D1 is often overexpressed in colorectal cancers (11,12,14). Amplification and overexpression of CDK4 is also seen in human cancers (11,12). Abnormalities in the expression of CDIs and in the retinoblastoma (Rb) gene, which plays a crucial role in controlling the G1–S transition, are described below. The proteins CDC25A and CDC25B, which are phos-
that inhibit cyclin D1 expression or activity, or the activity of lines also express high levels of p27K1p1, even during exponen-
tation (39). This regulation of p27 Kip1 appears to occur at a post-

-kinase activity, in both cell lines.

Fig. 2. Schematic diagram indicating that cyclin D1 and cyclin E, when bound to CDKs, stimulate the G1→S transition of the cell cycle. At elevated levels they also can induce (through unknown mechanisms) an increase in cellular levels of the p27Kip1 protein, thus providing feedback inhibition of the activities of these cyclins.

we transfected the MCF7 human breast cancer cell line and the MCF10F human non-tumorigenic mammary epithelial cell line with a vector containing the p27Kip1 cDNA to obtain derivatives that express increased levels of p27Kip1 (40). The increased expression of p27Kip1 in derivatives of both of these cell lines was associated with lengthening of the G1 phase, an increase in the doubling time, a decreased saturation density and a decreased plating efficiency. In the MCF7 cells, anchorage-independent growth and in vivo tumorigenicity were also suppressed. These effects were associated with decreased cyclin E-associated in vitro kinase activity, in both cell lines. Thus, breast cancer cells are still responsive to p27Kip1-mediated inhibition of cell growth despite the high basal level of this protein. These results suggest that therapeutic strategies that further increase the level of expression of p27Kip1 or mimic its activity might be useful in cancer therapy (40).

Curiously, we found that cancer cell lines and tumors that had high levels of p27Kip1 also frequently had high levels of cyclin D1 (28, 31–35). Furthermore, ectopic overexpression of cyclin D1 in esophageal (28) or mammary epithelial cell lines (31) was associated with increased expression of p27Kip1, and when an antisense cyclin D1 cDNA was introduced into either esophageal or colon cancer cells to reduce the expression of cyclin D1, this led to reduced levels of the p27Kip1 protein (22,23 and unpublished data). We also found that overexpression of cyclin E in mammary epithelial cells is associated with increased expression of p27Kip1 (33,41). Taken together, these findings suggest the existence of a feedback loop between cyclin D1 or cyclin E and p27Kip1, the purpose of which is to maintain a homeostatic balance between positive and negative regulators of the G1→S transition in the cell cycle (Figure 2). The increased levels of p27Kip1 in cancer cells might protect these cells from potentially toxic effects of increased expression of cyclin D1 and or cyclin E (10,29). This regulation of p27Kip1 appears to occur at a post-translational level which is consistent with the fact that the regulation of its expression is usually regulated at this level by a ubiquitin-protesome mediated mechanism (29). There is evidence that one mechanism by which cancer cells are protected from the inhibitory effects of p27Kip1 is to sequester this protein in the cytoplasm to prevent it from inhibiting

**Paradoxical overexpression of tumor suppressor genes**

**Studies on p27Kip1**

As discussed above, according to the current paradigm carcinogenesis is associated with activation of oncogenes and decreased expression of tumor suppressor genes. Therefore, we were surprised to find relatively high levels of expression of the protein p27Kip1 in a series of human esophageal cancer cell lines (28). This protein inhibits cell cycle progression by binding to and inhibiting the activities of cyclin/CDK complexes (Figure 1B), especially cyclin E/CDK2 and, therefore, it is a putative tumor suppressor gene (29). In addition, genetically engineered mice with reduced expression of p27Kip1 display increased sensitivity to carcinogen-induced tumor formation, even if only one allele is inactivated (30). We found that several human colon and breast cancer cell lines also express high levels of p27Kip1, even during exponential growth, but this protein is expressed at low levels in three normal human mammary cell lines (31–35).

Furthermore, we found that whereas in normal mammary epithelial cells the level of the p27Kip1 protein varies during the cell cycle, in breast cancer cell lines the level can remain high throughout the cell cycle (34). The high level of p27Kip1 in these cancer cells is not simply an artefact of cell culture, since we and other investigators have found that p27Kip1 is also expressed at relatively high levels in a subset of primary human breast and colon cancers (34–38). It is also over-expressed in small-cell carcinomas of the lung, despite their high degree of malignancy (39).

The increased expression of p27Kip1 in cancer cells seems paradoxical, especially because mutations in this gene have not been found or are extremely rare in various cancers (29). A possible explanation for the paradoxical increase in p27Kip1 in some cancer cells is that they have become refractory to the inhibitory effects of this protein. To address this question,
cancers of the breast, colon, stomach, prostate and oral (44,45). A striking
association with high grade (poorly differentiated) tumors and cyclin D1 protein and cyclin D1-associated kinase activity.

decreased expression of this protein, and that this decrease is the level of pRb by ~70% and also decreased the levels of the
studies indicate that another subset of human cancers displays of pRb (44). Treatment of HC116 cells with AS-Rb decreased
display relatively high levels of the p27 Kip1 protein, including nucleotide (AS-Rb) targeted to
esophageal, breast, colon and small-cell lung cancers, recent colon carcinoma cell line that expresses a relatively high level
we wondered if p27Kip1 played a role in the differentiation of these cancers. Therefore, we examined the effects of stably overexpressing high levels of p27Kip1 in the human colon cancer cell line HT29, which can be induced to undergo differentiation in response to treatment with sodium butyrate (42). We found that the p27Kip1 over-expressor clones displayed an increase in the amount of the p27Kip1 protein in cyclin E/CDK2 immunoprecipitates and a corresponding decrease in cyclin E-associated kinase activity, when compared with vector control clones, providing evidence that the overexpressed protein was functional. Clones with a high level of p27Kip1 displayed partial growth inhibition in monolayer culture and a decrease in plating efficiency, even though they expressed increased levels of the cyclin D1 protein. Using alkaline phosphatase expression as a marker, we found that the p27Kip1 overexpressor clones displayed a 2–3-fold increase in sensitivity to induction of differentiation by 2 mM sodium butyrate. In contrast with these results, derivatives of HT29 cells that stably overexpressed p21Cip1/Waf1 displayed decreased sensitivity to the induction of differentiation (42). These results may explain why decreased levels of p27Kip1 in certain human cancers are associated with high grade tumors. They also provide further evidence that therapeutic strategies that cause an increase in the level of p27Kip1 may be useful in cancer therapy. Since there already exist several agents that can increase the expression of p27Kip1 in specific cell types, including TGFβ, IFN-β, IFN-γ, cAMP agonists and rapamycin (29), in specific cell systems, this approach may be clinically feasible. Furthermore, adenoviral p27Kip1 gene transfer can induce apoptosis in several types of cancer cell lines (for review see ref. 29).

Paradoxical increase in the Rb protein in colorectal cancer

The protein encoded by the Rb gene, pRb, normally plays a key role as a negative regulator of the G1–S transition in the cell cycle by binding the transcription factor E2F and preventing it from activating the transcription of genes required for the S phase (11,12). The Rb gene is inactivated in a variety of human cancers, but in colorectal carcinomas there is frequently increased expression of this gene (43,44). This is paradoxical in view of the known role of Rb as a tumor suppressor gene. In a recent study we compared the levels of expression of pRb in normal human colorectal mucosa, adenomatous polyps, and carcinomas by immunohistochemistry (44). We found that there was a progressive increase in the expression of pRb during the multistage process of colon carcino-
genesis. Thus, during the transition from normal mucosa to adenomatous polyps to carcinomas there was a progressive increase in pRb expression. In vitro studies were also done to examine the phenotypic effects of an antisense oligodeoxy-nucleotide (AS-Rb) targeted to Rb mRNA in the HCT116 colon carcinoma cell line that expresses a relatively high level of pRb (44). Treatment of HC116 cells with AS-Rb decreased the level of pRb by ~70% and also decreased the levels of the cyclin D1 protein and cyclin D1-associated kinase activity.

This finding is consistent with other evidence of the existence of a feedback regulatory loop between Rb and cyclin D1 (44,45). A striking finding was that AS-Rb inhibited the growth of HCT116 cells and induced apoptosis. Reporter assays indicated an ~17-fold increase in E2F activity. Furthermore, we could mimic the growth-inhibitory and apoptosis-inducing effects of AS-Rb by simply ectopically overexpressing E2F in HCT116 cells. These findings suggest that the increased expression of pRb in colorectal carcinoma cells may provide a homeostatic mechanism that protects them from growth inhibition and apoptosis, perhaps by counterbalancing the potentially toxic effects of excessive E2F. There is, indeed, evidence that the majority of colon tumors have high E2F activity (46). This could reflect the effects of activating mutations in the k-ras oncogene, increased expression of cyclin D1, or other factors that affect E2F levels and/or activity.

We found that transfection of our As-Rb into WI38 human lung fibroblasts stimulated rather then inhibited growth (44), which is consistent with previous evidence that in several cell types pRb acts as a growth inhibitor. The seemingly paradoxical effects found in colon cancer are not unique since there is evidence that subsets of human cancers and leukemias can also display increased expression of pRb, and that pRb can protect bladder cancer cells, osteosarcoma cells and hepatic carcinoma cells from apoptosis induced by various agents (44,47). It is also apparent that, whereas E2F can act as an oncogene in some cell systems, in others it can induce apoptosis (44,48). Thus, the effects of altered expression of pRb and E2F, like that of numerous other oncogenes or tumor suppressor genes, is highly context dependent. This subject is discussed in greater detail below. The mechanism by which pRb expression is upregulated in some human cancers is not known. It has been suggested that in some cases this may be due to loss of expression of p16INK4a, since there appears to be a homeostatic feedback regulatory loop between pRb and p16INK4a (44,49,50).

Paradoxical increases in other inhibitors of the cell cycle

As described above, the CDI p27Kip1 is often expressed at relatively high levels in human cancer cells. High levels of expression of another CDI, p21WAF1, have also been seen in some human tumors, including glial tumors (51), non-small cell lung carcinomas (52), leiomyosarcomas (53) and breast carcinomas (54). Curiously, in breast cancers high p21WAF1 expression was associated with high tumor grade and a poor prognosis (54). In pancreatic cancer cells there was a correlation between high expression of cyclin D1 and p21WAF1 (55). In addition, cyclin D1 can induce increased expression of p21WAF1 through an E2F mechanism (56). This provides another example of a homeostatic feedback mechanism that is retained in many tumors.

Human tumors often display loss of expression of the CDI and tumor suppressor p16INK4a, either because of mutations in the gene or transcriptional silencing due to hypermethylation
**Disorders in cell circuitry**

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## YIN/YANG homeostatic feedback loops in cell cycle circuitry

<table>
<thead>
<tr>
<th>YANG</th>
<th>YIN</th>
</tr>
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<tbody>
<tr>
<td>↑ cyclin D1</td>
<td>↑ p27, p21, Rb</td>
</tr>
<tr>
<td>↑ cyclin E</td>
<td>↑ p27</td>
</tr>
<tr>
<td>↑ mitogens, ras, raf</td>
<td>↑ p21</td>
</tr>
<tr>
<td>↓ p16</td>
<td>↓ Rb</td>
</tr>
<tr>
<td>↓ cyclin D1</td>
<td>↓ Rb</td>
</tr>
<tr>
<td>↓ cyclin D1, mdm2</td>
<td>↓ p53</td>
</tr>
</tbody>
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**Fig. 3.** Examples in which a factor which enhances growth (‘Yang’) leads to an increase in the expression of a factor that inhibits growth (‘Yin’) and vice versa. These effects are often cell type specific. For additional details see text and related references. For the effect of p53 on cyclin D1 see Chen *et al.* (59).

(57). However, recent studies indicate that subsets of gastric and colon cancer can display increased expression of the p16 Ink4a protein (H. Yamamoto, personal communication). High levels of expression of p16 Ink4 have also been seen in human neuroblastoma cell lines (58). The significance of this finding remains to be determined. It is of interest that loss of expression of pRb is often associated with increased expression of p16 Ink4, suggesting the existence of a homeostatic feedback loop between these two proteins, as discussed above (44,49,50).

### Overview: the role of homeostasis in carcinogenesis, when Yin meets Yang

The above studies indicate that in several types of human cancer there can be an increase in the expression of the tumor suppressor genes p27 Kip1, p21 WAF1, p16 Ink4, or Rb. As discussed above, this may reflect, at least in part, the existence of homeostatic feedback loops in cell circuitry that maintain an appropriate balance between growth promoting and growth inhibitory factors. Figure 3 lists additional examples of what appear to be homeostatic feedback loops in pathways of signal transduction, i.e. examples of a ‘Yin/Yang’ phenomenon in which a growth enhancing factor induces a growth inhibitory factor or visa versa. In addition, it is now apparent that the biologic effects of oncogenes and tumor suppressor genes are highly context dependent (10,60). Examples include the ability of an activated ras gene or the transcription factor E2F to either enhance apoptosis or induce malignant cell transformation, depending on the cell system (60). Furthermore, the biologic effects of several oncogenes depend on their level of expression. For example, moderate overexpression of cyclin D1 can enhance cell growth but a high level of expression can be toxic to cells (61). The biologic effects of various protein kinases are also dependent on the cell context and their level of expression. We encountered this phenomenon in our studies on the biologic effects of specific isoforms of protein kinase C (62) and their roles in signal transduction (63).

Table 1 illustrates the remarkable diversity in function of the known oncogenes and tumor suppressor genes. It should also be emphasized that many of the respective proteins interact with each other in complex networks, rather than simple linear pathways, that display cross-talk and negative or positive feedback loops, analogous to electronic circuits (10,64–66), and the various pathways of signal transduction can be thought of as interconnecting ‘modules’ (66). Therefore, the accumulated effects of the multiple mutations in cancer cells leads to bizarre types of circuitry, i.e. circuits which were not present in the original parental cell, or in any other normal cell type. As a consequence, certain proteins in a cancer cell function within a novel context, since they are linked, either upstream or downstream, to proteins they are not linked to in normal cells. For similar reasons, an increase, decrease or loss of a given protein in a tumor cell might have a different ‘meaning’ to a cancer cell than to a normal cell, and the experimental re-introduction of a deleted protein into a cancer cell might exert effects different from those that occur when the same protein is present and expressed in normal cells.

This formulation can help to explain certain otherwise unexpected experimental results, and may also provide reason for optimism in the design of cancer-specific therapeutic agents. It has always seemed puzzling why the introduction of a single wild-type tumor suppressor gene, like p53, Rb or APC, into malignant tumor cells that carry multiple mutations can profoundly inhibit growth or induce apoptosis and/or inhibit tumorigenicity (67–69). If, according to the current paradigm, these cells originally evolved into a malignant tumor through the stepwise acquisition of several mutations, then the correction of one of these mutations should have only a small inhibitory effect. We believe that these results reflect the altered or bizarre circuitry of cancer cells, and refer to this phenomenon as ‘gene hypersensitivity’ (10). In our studies on cyclin D1 we encountered another effect which also seemed puzzling. As mentioned above, we found that stable expression of an antisense cyclin D1 cDNA in a human esophageal cancer cell line, which carries an amplified cyclin D1 gene and expresses high levels of cyclin D1, depressed cyclin D1 expression, and this was associated with a dramatic reversion of the cells towards a more normal phenotype (22). Nevertheless, the residual level of cyclin D1 protein expression in the reverted cells was considerably higher than in other rapidly growing and highly tumorigenic cells in which cyclin D1 was not amplified or overexpressed. These findings suggest that during the original evolution of these cancer cells they became ‘addicted’ to cyclin D1 (10) and, therefore, require high levels of this protein to maintain their cancer phenotype. A possible explanation is that these cancer cells express relatively high levels of proteins that counteract the effects of cyclin D1, for example Rb or one of the CDIs. Thus, even only a partial decrease in cyclin D1 in these cells would alter the stoichiometry between it and the respective inhibitory proteins, thus resulting in net inhibition of cell growth (10). If this explanation is correct, then cancer cells that are addicted to cyclin D1 might be unusually susceptible to drugs that block the action...
of cyclin D1. This general model might also apply to other genes that are amplified and/or overexpressed, or constitutively activated, in cancer cells. Indeed, it has been shown that pancreatic cancer cells that carry a mutated and activated k-ras gene are more dependent on the function of the k-ras gene for growth than pancreatic cancer cells that do not carry this mutation (70). Another example of gene addiction is the finding that erB-2 antisense oligonucleotides inhibit the proliferation of breast carcinomas cells with erB-2 amplification but have no specific effect on breast cancer lines that do not have amplification of erB-2 (71). The bizarre circuitry of cancer cells and the phenomena of gene hypersensitivity and gene addition could be the long sought Achilles’ heel of cancer cells. Indeed, they might explain why tumor cells are often more susceptible to the induction of apoptosis than normal cells by some of the currently employed cancer chemotherapy agents.

The concept of homeostasis is pervasive in biologic systems and dates back to the 19th century physiologist Claude Bernard who emphasized the constancy of the ‘interior milieu’ of the body in the face of an ever-changing exterior environment. The term itself was first used by Walter B. Cannon in the 1930s who emphasized the role of the autonomic nervous system in maintaining steady states within the body (72). Subsequent studies of the endocrine system provided further examples. With the more recent elucidation of biochemical pathways of biosynthesis and energy metabolism and current studies on pathways of signal transduction, the concept of homeostasis has been extended to intracellular mechanisms. It seems likely that the principal of homeostasis is also maintained during the process of multistage carcinogenesis, as discussed above. This seems reasonable since, despite its numerous abnormalities, the cancer cell must coordinate highly complex functions in order to survive and replicate. Therefore, the clonal evolution theory of cancer, proposed by Nowell (73), and the current paradigm of oncogenes and tumor suppressor genes (4), requires modification. The multistage process of carcinogenesis does not simply involve the step-wise activation of growth-promoting oncogenes and inactivation of growth inhibitory tumor suppressor genes. The regulatory circuitry of the evolving population of tumor cells must adapt to the stochastic occurrence of these mutations, some of which might on their own inhibit growth or cause apoptosis. Presumably this occurs through homeostatic feedback mechanisms, like those described above, and/or cell selection, thus maintaining a homeostatic balance that favors optimal growth and viability. This concept could help to explain the long latent period in carcinogenesis and the complex and heterogeneous phenotypes of cancer cells. It also has implications with respect to novel approaches to cancer chemoprevention and therapy, because of the bizarre circuitry that results from these alterations and the phenomena of gene hypersensitivity and gene addition, as discussed above.

The concept of cancer as a global disturbance of the network of regulatory circuitry within cells also has implications with respect to the limitations of the current approaches used for characterizing the genotypes and phenotypes of specific cancers. Currently, this is often done by analyzing a few genes, transcripts or proteins. The recent development of microarray methods (74) and proteomics markedly expands our ability to assess complex profiles of gene expression in cancer cells and, therefore, are major advances. However, these methods do not provide a dynamic view of the actual circuitry of cancer cells. A challenging future goal is to develop novel methods to assess this circuitry in living cells, and also to develop mathematical models (for example, see refs 64–66) for analyzing the complex networks and their interactions. Hopefully, the insights obtained from this new level of analysis will provide even more powerful approaches to cancer prevention and treatment.

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