Review Article

Mechanisms of Disease

Rules for Making Human Tumor Cells

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THE development of cancer in humans involves a complex succession of events that usually occur over many decades. During this multistep process, the genomes of incipient cancer cells acquire mutant alleles of proto-oncogenes, tumor-suppressor genes, and other genes that control, directly or indirectly, cell proliferation. Different combinations of these mutant alleles are found in the genomes of the many distinct types of human cancer as well as in different cancers from the same tissue. An ever-increasing number of these genes have been shown to make contributions to the distinct steps involved in neoplastic transformation. The complexity of these observations provokes the question of whether these genes and the more than 100 distinct types of human cancer can ever be rationalized in terms of a small number of underlying biologic and biochemical principles. Recent successes in the experimental transformation of human cells indicate that the disruption of a limited number of cellular regulatory pathways is sufficient to impart a tumorigenic phenotype to a wide variety of normal cells. These results, in turn, suggest a series of genetic and cellular principles that may govern the formation of most, if not all, types of human cancers.

MULTIPLE ALTERATIONS IN THE GENOMES OF CELLS

During the past 25 years, cancer researchers have enumerated a bewildering array of phenotypes and have catalogued thousands of molecular alterations associated with the malignant state. The rate at which these molecular markers are being identified continues to increase rapidly. Indeed, the recent use of transcriptional profiling to analyze human cancer cells has accelerated the tempo at which descriptions of cancer-related genes appear in the literature.[1-6]

Many of these studies have been motivated by the notion that the complex phenotypes of cancer cells will ultimately be explained by discovering associated changes in the genomes of these cells. There is also the hope of understanding the complex process of neoplastic transformation at the cellular level in terms of a small number of underlying genetic changes. Identification of the genetic changes in cancer cells and of the proteins that these changes affect promises to provide diagnostic and prognostic markers as well as molecular targets for therapeutic intervention.

Simple Transforming Systems

For those who believe in the simplification and rationalization of the cancer process, the actual course of research on the molecular basis of cancer has been largely disappointing. Rather than revealing a small number of genetic and biochemical determinants operating within cancer cells, molecular analyses of human cancers have revealed a bewilderingly complex array of such factors.[7,8] In the early 1970s, research on transforming retroviruses indicated that the neoplastic phenotype could be conferred on virus-infected cells by the actions of a limited number of genes. For example, the actions of a single virus-borne gene allowed Rous sarcoma virus to transform the chicken cells that it infected.[9] An independent line of research, which involved the transfer of genes from tumor cells into established rodent cells, identified specific oncogenes in the genomes of the tumor cells that could transform these recipient cells.[10-17] In these cases, the cancer-causing genes were found to be mutant versions of normal growth-controlling genes, which came to be called proto-oncogenes.[18]

Collaborating Oncogenes in Rodent Cells

In fact, the rodent cells used as recipients in these gene-transfer studies were not completely normal, since they had previously undergone immortalization in culture and thus had acquired the ability to proliferate indefinitely.[19-21] When truly normal rodent cells — specifically, those recently prepared from rat embryos (primary cells) — were tested, single oncogenes failed to induce transformation. Ultimately, these experiments revealed that at least two oncogenes needed to be introduced into the recipient cells to prompt them to enter a tumorigenic state.[22-24] In a general

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sense, these observations indicated that under most conditions, the conversion of normal cells into tumor cells requires multiple mutant genes.

The transformation of cultured primary cells from rodents involved the introduction of two collaborating oncogenes, such as *ras* and *myc*.[22,23] These experiments were subsequently extended in studies with transgenic mice, in which these two oncogenes were placed under the control of a transcriptional promoter that ensured their expression in certain tissues.[25,26] In mice carrying in their germ line either a *ras* or a *myc* transgene under the control of mammary- or prostate-specific promoters, dysplasia of mammary or prostate tissues developed at high rates. However, when mice carrying *ras* transgenes were bred with carriers of *myc* transgenes, cancers developed in the resulting double-transgenic mice; this synergy of the actions of *myc* and *ras* in vivo provided strong support for the oncogene collaboration that had been observed earlier in cultured cells. However, the kinetics of tumor development in these doubly transgenic mice indicated that the two oncogenes, expressed together in specific tissues of the mice, were still not sufficient to effect full tumorigenic transformation of the cells in these tissues. Indeed, further alterations — ostensibly, the mutation of additional genes — appeared to be required for neoplastic transformation in these animal models.

Further Complexity in the Transformation of Human Cells

Indications that the neoplastic transformation of human cells is even more complex than that of animal cells came from numerous attempts to transform cultured normal human cells into tumor cells by introducing *ras*, *myc*, and other oncogenes. Invariably, such attempts failed, whereas identical experimental protocols in rodent cells yielded large numbers of transformed, tumorigenic cells.[27,28] This dichotomy indicated that human and rodent cells respond very differently to introduced oncogenes and that human cells require an even greater number of genetic alterations than rodent cells for transformation to a neoplastic state. Indeed, subsequent attempts to transform primary human cells with combinations of oncogenes failed unless chemical or physical agents or stringent selection for rare immortalized variants was used.[29-32]

Without experimentally transformed human cancer cells, researchers were limited to the study of human cancer cells derived from tumor-biopsy specimens. In many cases, the cells from these tumors were adapted to growth in culture and were used to create tumor-cell lines.[33] Though useful for many studies, these human tumor cells, whether prepared directly from tumor samples or from cell lines, had limited usefulness for determining the genetic and biochemical rules governing the transformation of human cells. The available technology allowed only partial identification of the mutant genes (such as oncogenes and tumor-suppressor genes) in a cancer cell. Indeed, to this day, no one has sequenced the entire genome of a neoplastic human cell or has determined the entire set of mutant alleles that coexist within such a genome. In sum, these difficulties have made it impossible to identify a set of genetic alterations that are required, in concert, to program the neoplastic phenotype of a human cancer cell.

Gene Silencing by Methylation of DNA

Even if rapid sequencing of an entire cancer-cell genome were possible, as it surely will be sometime during the next decade, yet another factor will stand in the way of determining definitively the number of changes that are required for neoplastic transformation of a human cell. In addition to the presence of dominantly acting oncogenes, the functional loss of proteins encoded by tumor-suppressor genes also contributes, to an equal extent, to the neoplastic phenotype.[34-36] In many cases, inactivation of tumor-suppressor genes occurs through mutation or loss of a large portion of their genetic sequence.[37] However, recent evidence indicates that an equally effective mechanism of eliminating the function of tumor-suppressor genes entails the methylation of nucleotides in the promoter sequences that control the expression of these genes.[38]

Such methylation occurs in mammalian cells through the actions of DNA methylases that attach methyl groups to the cytidine residues of cytidine–guanosine (CpG) sequences in the cell genome. When CpG methylation occurs in an unregulated fashion, as it frequently does during tumor progression, the expression of various genes may be silenced.[39] DNA sequencing fails to detect these epigenetic alterations, and therefore even exhaustive sequencing of tumor-cell genomes will inevitably underestimate the number of genes that have a central role in programming the phenotype of a tumor cell. Moreover, expression profiling and functional genomics will also fail to identify the small number of altered, aberrantly functioning control genes responsible for orchestrating the malignant phenotype, since complex regulatory circuits are interposed between these control genes and the thousands of other genes whose expression they regulate.

THE GENETIC HISTORY OF HUMAN CANCER

The Complexity of Tumor Pathogenesis

The various factors described above have frustrated efforts to elucidate the complex genetic and epigenetic alterations that affect the genomes of human cells and that are required in concert for malignant transformation. Epidemiologic analyses of the incidence

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with which specific types of cancer appear in the human population as a function of age provide some measure of the number of distinct changes that must occur for tumorigenesis to reach completion and hence suggest the complexity of the problem. Kinetic analyses have shown that in the case of most tumors, four to six rate-limiting events must occur before the tumor becomes clinically apparent.[40,41] Presumably, these critical events result from somatic mutations that have a low probability of occurrence with cell division. However, such analyses, like the others described above, provide only a minimal estimate of the number of genetic changes that must occur for cancer to erupt, since they register only the rate-limiting steps in tumor progression and overlook other steps that may be equally important but occur far more rapidly.

Colorectal Cancer

A more compelling analysis of the complexity of the genetic program of cancer development in humans has come from detailed studies of colorectal carcinoma, undertaken more than a decade ago by Vogelstein and colleagues.[42] These researchers catalogued the genetic alterations present in a series of colonic-tissue biopsy specimens representing the various histopathological stages from normal epithelium to frank carcinoma.[43,44] They found that the great majority of early adenomatous polyps carried mutant, inactivated forms of the tumor-suppressor gene *APC*. About half of the adenomas of intermediate size carried mutant, activated *ras* oncogenes. A portion of the adenomas of yet larger size also carried an alteration of a chromosome 18–associated tumor-suppressor gene. Finally, perhaps half of the advanced colon carcinomas showed evidence of mutational inactivation of the tumor-suppressor gene *TP53*.[44] These observations suggested a genetic path that leads, in a subgroup of colon carcinomas, to the neoplastic state. The nature and role of additional genetic alterations in these tumor cells remain undefined.[45] Nevertheless, these observations provide an important example of how the genetic history of human cancer can be traced.

Unfortunately, no other epithelial cancer has yet been described in such detail, and even this scheme fails to define the precise number and nature of the genetic alterations that are required in concert for normal cells to grow as tumor cells in humans. As a consequence, it remains possible that each tumor is unique and that the spectrum of genetic changes that culminate in human tumors is infinitely variable. According to this point of view, no underlying rules govern the formation of all types of human cancers, and as we discover more about the mutant genes present in these cancers, our understanding of the biology of these tumors will become a phenomenology of unlimited complexity.

A LIMITED NUMBER OF ACQUIRED PHENOTYPES IN ALL CANCERS

On the basis of several lines of recent research, we embrace an alternative point of view: that the pathogenesis of human cancers is governed by a set of genetic and biochemical rules that apply to most and perhaps all types of human tumors.[46] We believe that the identities of the mutant genes in human tumor cells will one day be conceptualized in terms of these underlying rules. As we discuss below, these rules reflect the operations of a few key intracellular regulatory circuits that operate in the majority of human cell types. Although we still do not fully understand the detailed operations of these regulatory circuits, experimental observations during the past several years make it possible to outline the basic rules governing the neoplastic transformation of normal human cells.[47]

Part of this evolution in thinking about the origins of cancer comes from numerous observations indicating that most, if not all, cancer cells seem to share a common set of biologic attributes — essentially, changes in cell physiology — termed "acquired capabilities."[46] These attributes include the ability of cancer cells to generate their own mitogenic signals, to resist exogenous growth-inhibitory signals, to evade apoptosis, to proliferate without limits (i.e., to undergo immortalization), to acquire vasculature (i.e., to undergo angiogenesis), and in more advanced cancers, to invade and metastasize.

Genetic Instability

We imagine that these acquired capabilities are sufficient to explain the malignant behavior that characterizes cancer cells. In addition, human cancer cells often reveal an additional attribute — genetic instability — that enables them to acquire the other attributes. The measured rate of mutation in normal human cells is so low that during the course of a person's lifetime, they may not acquire the full array of mutant alleles that are required to complete the progression to a highly neoplastic state.[48] This calculation implies that the genomes of preneoplastic cells must become unstable for tumor progression to proceed to completion over a period of several decades.[49] Indeed, even cursory examinations of human tumor-cell genomes usually reveal instability at the level of either the DNA sequence or the karyotype — an observation that helps support the notion that increased mutability is essential for the development of many types of cancer in humans.[45] Such increased mutability is acquired when the genes and proteins that ordinarily protect the genome by detecting and repairing damage in chromosomal DNA are inactivated. In addition, the cellular mechanisms (notably apoptosis) that usually eliminate cells with damaged DNA are often compromised in tumor cells; the result is the survival of a mu-

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tant cell and the possible outgrowth of a large population of its similarly mutated descendants.

Disrupted Regulatory Circuits

The catalogue of these acquired attributes and an additional characteristic — genetic instability — suggest that a limited set of governing principles may someday be used to explain the pathogenesis of cancer. Yet another indication that the process can be simplified comes from biochemical and molecular analyses of the regulatory pathways that have key functions in most types of normal human cells. Comparative studies of various types of human cancer cells indicate that the regulation of each of these pathways can be disrupted by a number of alternative genetic and epigenetic alterations.

The Retinoblastoma Protein

The pathway governed by the retinoblastoma protein (pRB) plays a central part in determining whether a cell will proceed through the G_1 phase of the cell cycle (Fig. 1).[50] This control circuit can be disturbed by several alternative genetic and biochemical mechanisms. In retinoblastomas, osteosarcomas, and small-cell lung carcinomas, the pRB protein is absent because of mutations that disable the *RB* gene.[51] In many cervical carcinomas, the pRB protein is sequestered and tagged for degradation by the E7 oncoprotein of type 16 and 18 human papillomaviruses.[52,53] In a diverse array of cancers, inactivation of the *p16*^{INK4A} gene, by genetic lesions or by methylation, disrupts the regulation of phosphorylation and causes functional inactivation of pRB.[54,55] In breast cancer, overexpression of cyclin D1 or cyclin E has the same outcome.[56,57] Indeed, the weight of evidence suggests that such alterations, all of which converge on the loss of growth suppression by pRB, exist in the majority of cancers in humans.[51]

The p53 Protein

The pathway controlled by the p53 tumor-suppressor protein is also altered in most, if not all, cancers in humans (Fig. 1).[58] In normal cells, p53 is responsible for temporarily arresting cell growth in response to certain types of molecular and biochemical damage until such damage can be repaired; other types of damage and physiologic stress act by way of the p53 protein to trigger a program of apoptosis (cell suicide), which eliminates the damaged cell.[58-60] Indeed, the *TP53* gene is mutated in as many as half of all human tumors; by now, more than 15,000 mutant *TP53* alleles have been sequenced and have been found to carry inactivating mutations.[61,62] In some tumors without any signs of *TP53* mutation, the p53 antagonist, the human form of murine double minute 2 protein (HDM2), is overexpressed, driving the premature degradation of p53 protein.[63-66] In other tumors, the gene encoding the HDM2 antagonist, *p19*^{ARF}, is deleted or its expression is suppressed by methylation; its absence permits HDM2 to drive the degradation of p53.[54,55] As before, a diverse array of biochemical and genetic changes converge on a common biochemical, and thus cellular physiologic, outcome.

The drive to eliminate functional p53 in evolving, preneoplastic cells suggests that there is pressure in these cells to reduce or eliminate the actions of proapoptotic proteins. When present, these proteins together orchestrate the cell-suicide program, and their continued presence in functional form in incipient cancer cells is a major obstacle to tumor formation. Elimination of functioning p53 appears to be sufficient to inactivate the apoptotic machinery in many types of cancer cells.[67] In others, specific components of the apoptotic machinery are discarded or inactivated.[68-71]

Telomeres

Maintenance of telomeres, protective sequences that constitute the ends of chromosomes, also appears to be an important ingredient in the formation of most and probably all types of human cancers (Fig. 2).[72-75] Ongoing maintenance of telomeres is a prerequisite for the indefinite proliferation of cells — the phenotype of cell immortalization — and is clearly an intrinsic part of the neoplastic-growth program. In perhaps 90 percent of tumors in humans, the maintenance of telomeres and thus replicative immortality are achieved through reactivation of telomerase, the expression of which is suppressed in most normal human cell types[76]; in the remaining tumors, telomeres seem to be maintained by the actions of a telomerase-independent mechanism, termed "alternative lengthening of telomeres."[77]

Mitogenic Stimulation

Another biochemical pathway reveals that normal and neoplastic cells differ in their dependence on mitogenic stimulation. The proliferation of normal cells appears to depend on the presence of growth factors in their surroundings; in the absence of these external signals, cells will not make the commitment to proliferate. Cancer cells, in stark contrast, have a strongly reduced dependence on external mitogenic stimulation.[78] This acquired independence derives from the activities of oncogenes that generate constitutive mitogenic signals.[8] For example, the *ras* oncogene, which is found in its mutant, activated state in perhaps one quarter of all tumors in humans, encodes a mutant protein that releases a continuous stream of mitogenic signals into the cell cytoplasm, thereby obviating the need for a cancer cell to encounter growth factors in its external environment (Fig. 3).[82] A similar stream of growth-stimulation signals can result from alter-

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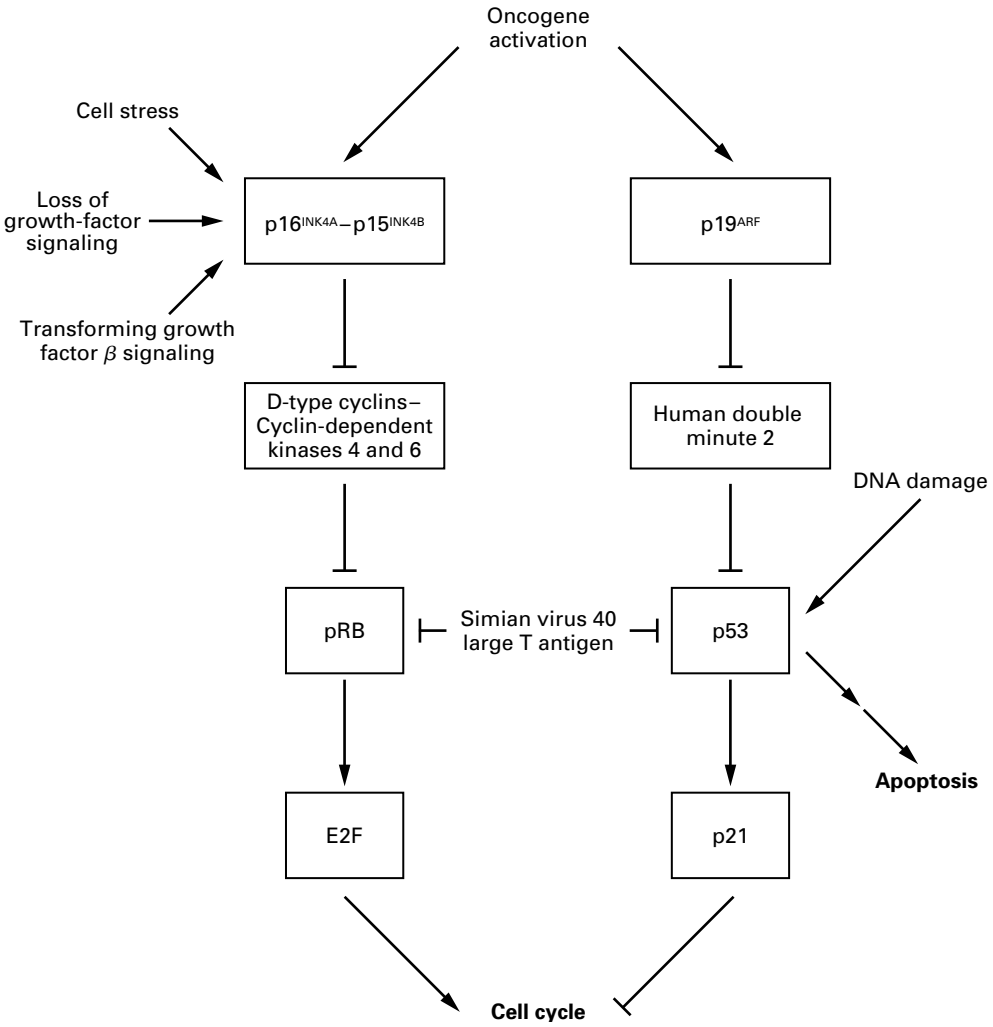

Figure 1. The Retinoblastoma Protein and p53 Tumor-Suppressor Pathways.

The retinoblastoma protein (pRB) and p53 tumor-suppressor protein have central roles in regulating the cellular response to external signals. Each of these tumor-suppressor proteins is itself regulated by a series of other proteins that together constitute a molecular pathway. Mutations or alterations in expression for each component of these pathways have been described in human cancers, suggesting that disruption of the regulation of these pathways can occur at multiple points. E2F is a family of transcription factors that regulates cell-cycle progression. Arrows represent activating interactions, and barred lines indicate inhibitory interactions. The pathway leading to apoptosis involves many steps that are not shown.

ations in growth-factor receptors on the cell surface. In a diverse array of human tumors, these receptors have been found to be overexpressed or structurally altered. For example, K-*ras* mutations are highly prevalent in lung, pancreatic, and colon cancers[83,84]; amplification of the receptor tyrosine kinase HER2/neu occurs in a considerable proportion of aggressive breast cancers[85,86]; and mutations of the BRaf protein, which lies immediately downstream of Ras (Fig. 3), are found in up to 66 percent of melanomas.[87] It seems that a signaling pathway involved in receiving and processing biochemical signals is disturbed in the majority of cancers in humans.

Angiogenesis

Yet another biologic commonality among diverse tumors in humans is their angiogenic powers.[88] Incipient tumors cannot grow to sizes greater than 2 mm in diameter unless they succeed in acquiring access to the circulatory system.[89,90] They do so through their ability to release angiogenic signals, which attract and stimulate endothelial cells. The latter, in turn, con-

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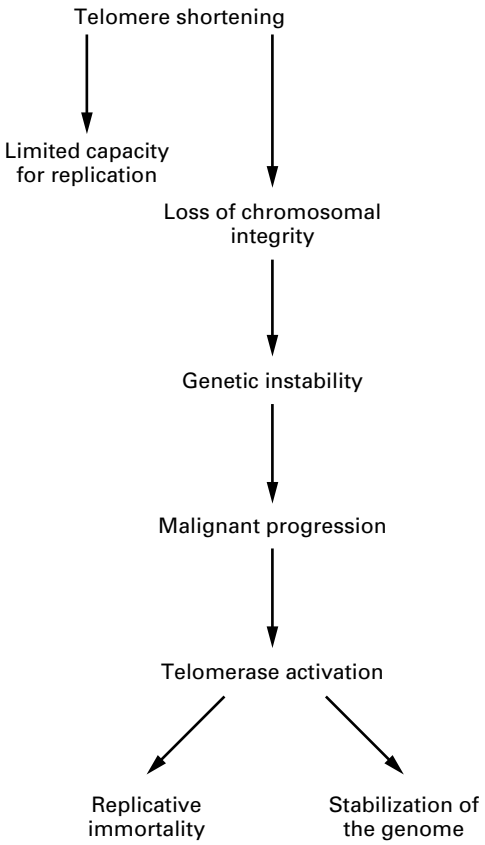

Figure 2. Telomeres, Telomerase, and Cancer.

The shortening of telomeres has a dual role in the development of cancer. Telomere attrition limits the replicative life span of a cell; however, such shortening eventually prevents telomeres from adequately protecting the ends of chromosomes from damage and further degradation. Studies in human cells and in animal models reveal that widespread cell death occurs at this point, termed "crisis," which is associated with substantially increased chromosomal instability. Such karyotypic instability not only drives the selection of cells that reactivate telomerase but also promotes the acquisition of other mutations that may participate in further oncogenic progression.[72]

struct capillaries within the tumor mass, and the capillaries forge direct connections with the existing vasculature of the host, providing nutrients and oxygen to the tumor cells and facilitating the evacuation of their metabolic wastes.

All solid tumors achieve angiogenesis by secreting proangiogenic factors, notably vascular endothelial growth factor or basic fibroblast growth factor, and by down-regulating the expression of antiangiogenic proteins, such as thrombospondin-1.[89,91] However, this is a simplified view of what is actually a far more complex process, one that involves an array of signaling proteins and a number of distinct stromal cell

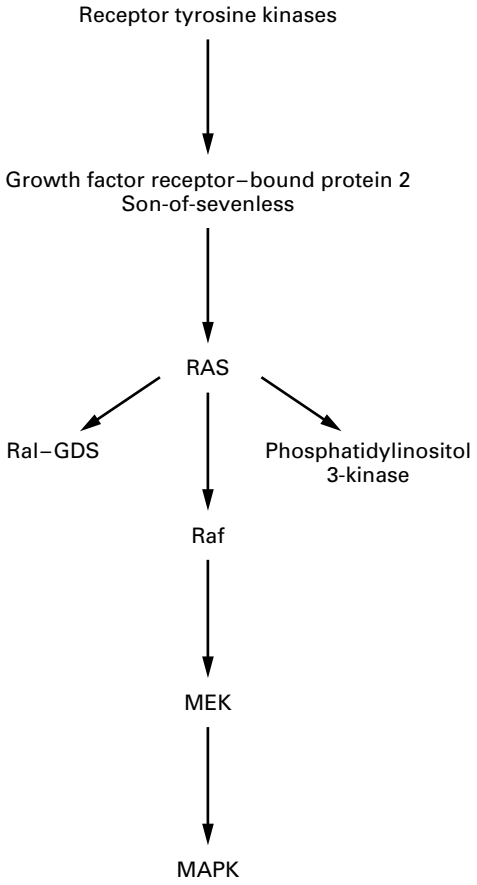

Figure 3. Mitogenic Signaling Pathways.

Many oncogenes activate mitogenic signaling pathways, such as those controlled by receptor tyrosine kinases and Ras. Mutations or alterations in the expression of each of the members of this pathway are associated with cancer; in most cases, such changes lead to activation or unrestrained functioning of the pathways. Normally, the binding of a growth factor to a receptor tyrosine kinase recruits and activates the adaptor proteins growth factor receptor–bound protein 2 and son-of-sevenless, which in turn recruit the small guanosine triphosphate–binding protein Ras. This association activates a cascade of serine–threonine kinases (Raf and mitogen-activated and extracellularly activated kinase [MEK]), culminating in the activation of a mitogen-activated protein kinase (MAPK). MAPKs then move to the nucleus, where, by phosphorylating transcription factors, they modulate the expression of a wide range of genes involved in cell growth and survival. Ras has a number of effectors (not all shown) other than MAPK,[79] many of which may also impinge on the malignant phenotype.

A second pathway activated by growth factors is the phosphatidylinositol 3-kinase pathway, which activates the serine–threonine kinase Akt (also known as protein kinase B [not shown]).[80] Inactivation of the lipid phosphatase called phosphatase and tensin homologue deleted on chromosome 10 (PTEN) also results in activation of this pathway, and inherited loss of PTEN confers susceptibility to many types of cancer.[81] Ras activation can also lead to activation of phosphatidylinositol 3-kinase, indicating that many connections unite these pathways. Ras can activate other signaling pathways, such as Ral–guanine nucleotide dissociation stimulator (Ral–GDS).

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types that collaborate with cancer cells to prompt the growth of new blood vessels. As with the other acquired capabilities, it is apparent that angiogenesis is a common attribute of all solid tumors and perhaps of certain types of hematopoietic cancers as well. Angiogenesis is probably regulated by a common molecular circuitry in many different types of cells, although the detailed design of this circuitry remains poorly understood.

EXPERIMENTAL EVIDENCE OF KEY REGULATORY PATHWAYS

The list of biologic and biochemical attributes shared by many if not all types of tumors, as discussed above, is persuasive evidence that a set of common rules governs the neoplastic transformation of a wide spectrum of human cells. Still, this evidence fails to address a critical question: How many distinct regulatory changes are required in aggregate to transform a normal human cell into a tumor cell?

Introduction of Genes into Cultured Cells

The above-mentioned biochemical changes — in the pRB, p53, and Ras pathways; in the telomere-maintenance system; and in the circuitry that regulates angiogenesis — may well include all the changes that are required for the neoplastic transformation of human cells. In that case, this list provides a compelling guide to the entire process of the transformation of human cells (Fig. 4). It is also possible that several other changes still awaiting discovery are required. An effective way of resolving these ambiguities involves the experimental genetic manipulation of human cells[47]: the genotype of cultured normal human cells can be changed in defined ways through the introduction of specific genes and subsequent determination of whether the introduced genetic elements are sufficient to prompt the cells to enter a tumorigenic state. Success in this undertaking should reveal the overall complexity of the neoplastic transformation of human cells.

Such experimental transformation of human cells became possible several years ago with the isolation of the *hTERT* gene, which encodes the catalytic subunit of telomerase.[92-94] Telomerase expressed by an experimentally introduced gene in human cells stabilized their telomeres and facilitated their immortalization.[95] Findings such as this one permitted subsequent demonstration of the neoplastic transformation of these cells.[96-101] The rationale behind these experiments came from studies in the early 1980s that showed that experimental transformation of normal rodent cells into tumor cells involved two distinct steps.[22,23] The first is cell immortalization, which is required to make cells susceptible to the second step — transformation by an oncogene, such as *ras*.[21,97]

The immortalization of cultured human cells is itself a process of some complexity. When normal human fibroblasts are grown in culture, they proliferate for 60 to 80 cell generations and then enter a nongrowing but viable state termed replicative senescence.[102] The introduction of genes encoding viral oncoproteins, such as the large T antigen of simian virus 40 (SV40), allows presenescent cells to circumvent senescence[103]; the large T oncoprotein accomplishes this task by sequestering and inactivating the two critical proteins encoded by tumor-suppressor genes: pRB and p53.[104]

Immortalization, Crisis, and Transformation

Having circumvented senescence, cells bearing the large T antigen can then continue to double an additional 10 to 20 times, at which point they enter the crisis state, in which the majority of them show karyotypic disarray and die by apoptosis.[105,106] Crisis clearly is provoked by telomere attrition in these cells (Fig. 2).[107] In culture, the telomeres of normal human cells shorten by 50 to 100 base pairs during each cell generation.[108] We now know that the timing of the onset of crisis is governed by the time required for the initially long telomeres of early-passage cells to erode and shorten until they are no longer able to protect the ends of chromosomal DNA. Emerging from populations of cells in crisis are rare variants (1 in 10 million cells) that have acquired the ability to proliferate indefinitely. These cells express the *hTERT* gene, which is strongly repressed in most lineages of normal human cells.[107] It thus becomes clear how ectopic expression of *hTERT* can ensure the immortalization of many normal cell types.[109-111]

Informed by these observations, we and others successively introduced the SV40 early region (which encodes both the large T and small t oncoproteins) and the *hTERT* gene into normal human fibroblasts and kidney cells.[97,98,100,101,112] Together, the proteins corresponding to these genes succeeded in immortalizing the cells. The subsequent introduction of a mutant *ras* oncogene into the immortalized cells resulted in their transformation to a tumorigenic state, as gauged by their ability to form tumors in immunocompromised animal hosts. The tumors were anaplastic, strongly angiogenic, and nonmetastatic. A significant percentage of the transformed human kidney cells retained a diploid karyotype, suggesting that overt changes in chromosomal configuration were not required to orchestrate cell transformation.[101,113,114] Additional studies in a wide variety of primary human cells have confirmed that these introduced genes are sufficient to transform mammary epithelial cells, epithelial cells from the small and large airways of the lung, prostate epithelial cells, ovarian epithelial cells, mesothelial cells, melanocytes, and neuroectodermal cells.[96,98-101,114] These observations suggest that the

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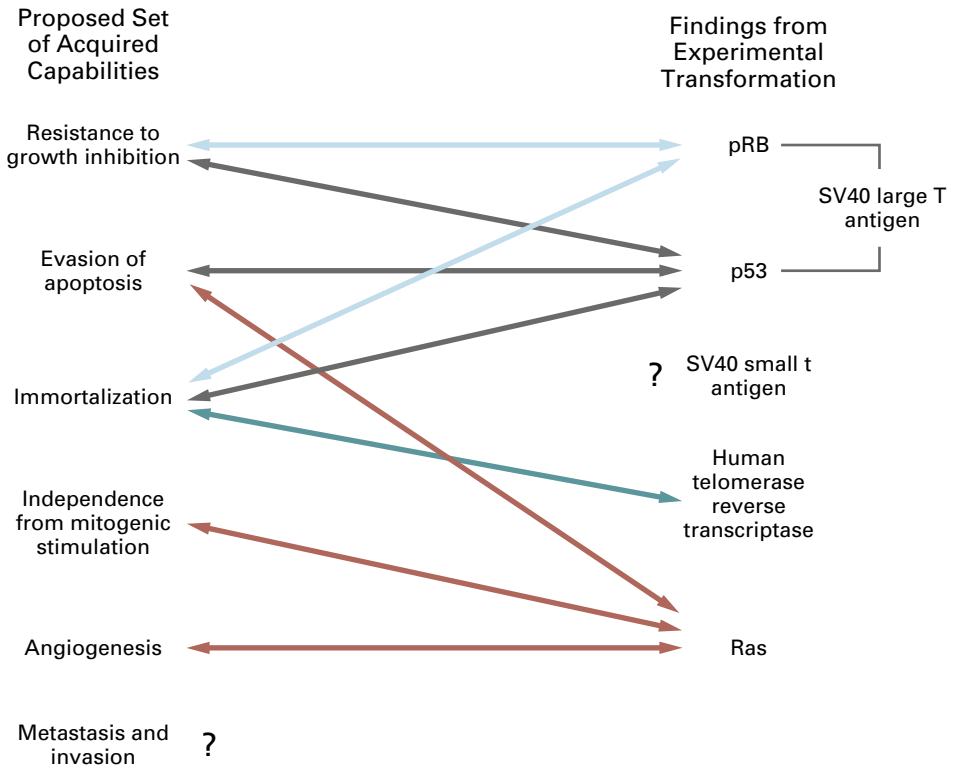

Figure 4. Acquired Capabilities, Molecular Pathways, and the Transformation of Human Cells: Emerging Rules That Govern Cancer Formation.

Most if not all cancerous cells exhibit several distinct biologic phenotypes that distinguish their behavior from that of normal cells. Recent advances in the experimental transformation of human cells, as well as detailed elucidation of several key pathways involved in the regulation of cell growth and proliferation, have begun to clarify the molecular machinery that programs cancer-cell behavior. These connections, though certainly incomplete, identify promising targets for the development of novel diagnostic and therapeutic agents. The molecular pathways that lead to metastasis and invasion remain unclear. pRB denotes the retinoblastoma protein, and SV40 simian virus 40. The SV40 large T antigen binds to and inhibits both pRB and p53.

genetic elements that were introduced into the cells provoked the cells' transformation by disturbing a small set of intracellular pathways, specifically those involving pRB and p53 (through the actions of large T antigen), the telomere-maintenance pathway, and the mitogenic signaling pathway.

In addition, these experiments show that the SV40 small t protein is also required for transformation to a tumorigenic state.[100,112] However, the precise biochemical and physiologic actions of this protein remain elusive.[115,116] The small t protein disturbs an abundant cellular enzyme called protein phosphatase 2A, which acts on a wide variety of substrates to remove phosphate groups from serine and threonine residues. Cancer-associated mutations have been reported in some of the genes that encode subunits of protein phosphatase 2A.[117-119] To date, knowledge of these mutations has shed little light on the biochemical actions of this enzyme that are relevant to cell transformation, since it is a complex enzyme and has numerous cellular phosphoprotein substrates and since the identities of the substrates that are critical to transformation are not yet known.[115,116]

EMERGING RULES GOVERNING HUMAN CANCER DEVELOPMENT

These diverse observations, taken together, embolden us to conclude that five distinct alterations suffice to transform normal human cells into tumor cells. Interestingly, only two or three of these changes seem to be required for rodent-cell transformation, explaining in retrospect the great difficulty experienced in early attempts to transform human cells.[47] Our formulation leaves open the question of whether these five changes are also necessary and sufficient for the spontaneous development of primary tumors within

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the body. A comparison between our list of five requisite changes and the list of five frequently observed alterations in the genomes of spontaneously arising human tumors shows great overlap (Fig. 4). Pathways involving the Ras, pRB, p53, and hTERT proteins are components of both lists. Angiogenesis is accomplished by this same set of genes experimentally introduced into human cells, as discussed above; the angiogenic phenotype derives in part from the actions of the Ras oncoprotein.[120,121] The exception here comes from the observed importance of the inactivation of protein phosphatase 2A in experimental transformation. Does alteration of some of the activities of this enzyme also occur in spontaneously arising human tumors, and if so, is it a change that is essential for transformation? The answers may be forthcoming over the next several years.

These results, incomplete as they may be, provide hope that similar conclusions and generalizations may one day be extended to describe the behavior of tumors encountered in the oncology clinic. If so, then the complex array of mutant and methylated genes found in human tumors may one day be understood in terms of this limited set of cellular regulatory pathways. Still unidentified are the genetic elements that control the final steps in tumor progression: invasion and metastasis. Even in this regard, we are optimistic that sometime during the next decade, these poorly understood processes will also be explained in terms of mechanisms that are common to widely different types of tumors. The science of cancer pathogenesis may one day become the study of a coherent set of rules and principles, rather than a phenomenology of unlimited complexity.

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REFERENCES

1. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999;286:531-7.
2. Yeang CH, Ramaswamy S, Tamayo P, et al. Molecular classification of multiple tumor types. Bioinformatics 2001;17:Suppl 1:S316-S322.
3. Bhattacharjee A, Richards WG, Staunton J, et al. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proc Natl Acad Sci U S A 2001;98:13790-5.
4. Garber ME, Troyanskaya OG, Schluens K, et al. Diversity of gene expression in adenocarcinoma of the lung. Proc Natl Acad Sci U S A 2001; 98:13784-9. [Erratum, Proc Natl Acad Sci U S A 2002;99:1098.]
5. Perou CM, Jeffrey SS, van de Rijn M, et al. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. Proc Natl Acad Sci U S A 1999;96:9212-7.
6. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-52.
7. Hunter T. Oncoprotein networks. Cell 1997;88:333-46.
8. *Idem*. Cooperation between oncogenes. Cell 1991;64:249-70.
9. Bishop JM. Viral oncogenes. Cell 1985;42:23-38.
10. Shih C, Padhy LC, Murray M, Weinberg RA. Transforming genes of carcinoma and neuroblastomas introduced into mouse fibroblasts. Nature 1981;290:261-4.
11. Krontiris TG, Cooper GM. Transforming activity of human tumor DNAs. Proc Natl Acad Sci U S A 1981;78:1181-4.
12. Perucho M, Goldfarb M, Shimizu K, Lama C, Fogh J, Wigler M. Human-tumor-derived cell lines contain common and different transforming genes. Cell 1981;27:467-76.
13. Pulciani S, Santos E, Lauver AV, Long LK, Robbins KC, Barbacid M. Oncogenes in human tumor cell lines: molecular cloning of a transforming gene from human bladder carcinoma cells. Proc Natl Acad Sci U S A 1982; 79:2845-9.
14. Shih C, Shilo BZ, Goldfarb MP, Dannenberg A, Weinberg RA. Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. Proc Natl Acad Sci U S A 1979;76:5714-8.
15. Balmain A, Pragnell IB. Mouse skin carcinomas induced in vivo by chemical carcinogens have a transforming Harvey-ras oncogene. Nature 1983;303:72-4.
16. Sukumar S, Notario V, Martin-Zanca D, Barbacid M. Induction of mammary carcinomas in rats by nitroso-methylurea involves malignant activation of H-ras-1 locus by single point mutations. Nature 1983;306:658-61.
17. Eva A, Aaronson SA. Frequent activation of c-kis as a transforming gene in fibrosarcomas induced by methylcholanthrene. Science 1983;220:955-6.
18. Bishop JM, Weinberg RA. Molecular oncology. New York: Scientific American, 1996.
19. Harvey DM, Levine AJ. p53 Alteration is a common event in the spontaneous immortalization of primary BALB/c murine embryo fibroblasts. Genes Dev 1991;5:2375-85.
20. Newbold RF, Overell RW, Connell JR. Induction of immortality is an early event in malignant transformation of mammalian cells by carcinogens. Nature 1982;299:633-5.
21. Newbold RF, Overell RW. Fibroblast immortality is a prerequisite for transformation by EJ c-HA-ras oncogene. Nature 1983;304:648-51.
22. Land H, Parada LF, Weinberg RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 1983;304:596-602.
23. Ruley HE. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature 1983;304:602-6.
24. Beug H, Kahn P, Doederlein G, Hayman MJ, Graf T. Characterization of hematopoietic cells transformed in vitro by AEV-H, a v-erbB-containing avian erythroblastosis virus. Hamatol Bluttransfus 1985;29:290-7.
25. Sinn E, Muller W, Pattengale P, Tepler I, Wallace R, Leder P. Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes in vivo. Cell 1987;49:465-75.
26. Thompson TC, Southgate J, Kitchener G, Land H. Multistage carcinogenesis induced by ras and myc oncogenes in a reconstituted organ. Cell 1989;56:917-30.
27. Stevenson M, Volsky DJ. Activated v-myc and v-ras oncogenes do not transform normal human lymphocytes. Mol Cell Biol 1986;6:3410-7.
28. Sager R, Tanaka K, Lau CC, Ebina Y, Anisowicz A. Resistance of human cells to tumorigenesis induced by cloned transforming genes. Proc Natl Acad Sci U S A 1983;80:7601-5.
29. Kang K-S, Sun W, Nomata K, et al. Involvement of tyrosine phosphorylation of $p185^{c\text{-}erbB2/neu}$ in tumorigenicity induced by x-rays and the *neu* oncogene in human breast epithelial cells. Mol Carcinog 1998;21:225-33.
30. Yoakum GH, Lechner JF, Gabrielson EW, et al. Transformation of human bronchial epithelial cells transfected by Harvey ras oncogene. Science 1985;227:1174-9.
31. Rhim JS, Jay G, Arnstein P, Price FM, Sanford KK, Aaronson SA. Neoplastic transformation of human epidermal keratinocytes by AD12-SV40 and Kirsten sarcoma viruses. Science 1985;227:1250-2.
32. Hurlin PJ, Maher VM, McCormick JJ. Malignant transformation of human fibroblasts caused by expression of a transfected T24 HRAS oncogene. Proc Natl Acad Sci U S A 1989;86:187-91.
33. Masters JR. Human cancer cell lines: fact and fantasy. Nat Rev Mol Cell Biol 2000;1:233-6.
34. DePinho RA, Jacks T. The laboratory mouse in cancer research. Semin Cancer Biol 2001;11:175-6.
35. Hakem R, Mak TW. Animal models of tumor-suppressor genes. Annu Rev Genet 2001;35:209-41.
36. Knudson AG. Two genetic hits (more or less) to cancer. Nat Rev Cancer 2001;1:157-62.

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37. Sager R. Tumor suppressor genes: the puzzle and the promise. Science 1989;246:1406-12.
38. Baylin S, Bestor TH. Altered methylation patterns in cancer cell genomes: cause or consequence? Cancer Cell 2002;1:299-305.
39. Laird PW, Jaenisch R. The role of DNA methylation in cancer genetic and epigenetics. Annu Rev Genet 1996;30:441-64.
40. Armitage P, Doll R. The age distribution of cancer and a multi-stage theory of carcinogenesis. Br J Cancer 1954;8:1-12.
41. Renan MJ. How many mutations are required for tumorigenesis? Implications from human cancer data. Mol Carcinog 1993;7:139-46.
42. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525-32.
43. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759-67.
44. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996;87:159-70.
45. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998;396:643-9.
46. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100: 57-70.
47. Hahn WC, Weinberg RA. Modelling the molecular circuitry of cancer. Nat Rev Cancer 2002;2:331-41.
48. Cahill DP, Kinzler KW, Vogelstein B, Lengauer C. Genetic instability and Darwinian selection in tumours. Trends Cell Biol 1999;9:M57-M60.
49. Loeb L. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 1991;51:3075-9.
50. Kaelin WG Jr. Functions of the retinoblastoma protein. Bioessays 1999;21:950-8.
51. Sellers WR, Kaelin WG Jr. Role of the retinoblastoma protein in the pathogenesis of human cancer. J Clin Oncol 1997;15:3301-12.
52. Munger K. The role of human papillomaviruses in human cancers. Front Biosci 2002;7:d641-d649.
53. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002;2:342-50.
54. Sharpless NE, DePinho RA. The INK4A/ARF locus and its two gene products. Curr Opin Genet Dev 1999;9:22-30.
55. Sherr CJ. The ink4a/ARF network in tumour suppression. Nat Rev Mol Cell Biol 2001;2:731-7.
56. Sicinski P, Weinberg RA. A specific role for cyclin D1 in mammary gland development. J Mammary Gland Biol Neoplasia 1997;2:335-42.
57. Jacks T, Weinberg RA. The expanding role of cell cycle regulators. Science 1998;280:1035-6.
58. Levine AJ. p53, The cellular gatekeeper for growth and division. Cell 1997;88:323-31.
59. Lowe SW. Activation of p53 by oncogenes. Endocr Relat Cancer 1999;6:45-8.
60. Jacks T, Weinberg RA. Cell-cycle control and its watchman. Nature 1996;381:643-4.
61. Soussi T, Gallou C, Beroud C. The p53 database. December 2001. (Accessed October 22, 2002, at http://p53.curie.fr.)
62. Harris CC. p53 Tumor suppressor gene: from the basic research laboratory to the clinic — an abridged historical perspective. Carcinogenesis 1996;17:1187-98.
63. Sanchez-Aguilera A, Sanchez-Beato M, Garcia JF, Prieto I, Pollan M, Piris MA. p14(ARF) nuclear overexpression in aggressive B-cell lymphomas is a sensor of malfunction of the common tumor suppressor pathways. Blood 2002;99:1411-8.
64. Polsky D, Bastian BC, Hazan C, et al. HDM2 protein overexpression, but not gene amplification, is related to tumorigenesis of cutaneous melanoma. Cancer Res 2001;61:7642-6.
65. Ho GH, Calvano JE, Bisogna M, et al. Genetic alterations of the p14ARF-hdm2-p53 regulatory pathway in breast carcinoma. Breast Cancer Res Treat 2001;65:225-32.
66. Freedman DA, Wu L, Levine AJ. Functions of the MDM2 oncoprotein. Cell Mol Life Sci 1999;55:96-107.
67. Shen Y, White E. p53-Dependent apoptosis pathways. Adv Cancer Res 2001;82:55-84.
68. Wang X. The expanding role of mitochondria in apoptosis. Genes Dev 2001;15:2922-33.
69. Yin XM, Oltvai ZN, Veis-Novack DJ, Linette GP, Korsmeyer SJ. Bcl-2 gene family and the regulation of programmed cell death. Cold Spring Harb Symp Quant Biol 1994;59:387-93.
70. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. Genes Dev 1999;13:2905-27.
71. Thornberry NA, Lazebnik Y. Caspases: enemies within. Science 1998; 281:1312-6.
72. DePinho RA. The age of cancer. Nature 2000;408:248-54.
73. Hahn WC, Meyerson M. Telomerase activation, cellular immortalization and cancer. Ann Med 2001;33:123-9.
74. Harley CB, Kim NW, Prowse KR, et al. Telomerase, cell immortality, and cancer. Cold Spring Harb Symp Quant Biol 1994;59:307-15.
75. Shay JW, Zou Y, Hiyama E, Wright WE. Telomerase and cancer. Hum Mol Genet 2001;10:677-85.
76. Kim NW, Piatyszek MA, Prowse KR, et al. Specific association of human telomerase activity with immortal cells and cancer. Science 1994;266: 2011-5.
77. Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. Nat Med 1997;3:1271-4.
78. McCormick F. Signalling networks that cause cancer. Trends Cell Biol 1999;9:M53-M56.
79. Shields JM, Pruitt K, McFall A, Shaub A, Der CJ. Understanding Ras: 'it ain't over 'til it's over.' Trends Cell Biol 2000;10:147-54.
80. Brazil DP, Hemmings BA. Ten years of protein kinase B signalling: a hard Akt to follow. Trends Biochem Sci 2001;26:657-64.
81. Di Cristofano A, Pandolfi PP. The multiple roles of PTEN in tumor suppression. Cell 2000;100:387-90.
82. McCormick F. GTP-binding proteins as oncogenes in human tumors. Environ Health Perspect 1991;93:17-8.
83. Ellis CA, Clark G. The importance of being K-Ras. Cell Signal 2000; 12:425-34.
84. Bos JL. ras Oncogenes in human cancer: a review. Cancer Res 1989; 49:4682-9. [Erratum, Cancer Res 1990;50:1352.]
85. Press MF, Jones LA, Godolphin W, Edwards CL, Slamon DJ. HER-2/neu oncogene amplification and expression in breast and ovarian cancers. Prog Clin Biol Res 1990;354A:209-21.
86. Ross JS, Fletcher JA. HER-2/neu (c-erb-B2) gene and protein in breast cancer. Am J Clin Pathol 1999;112:Suppl 1:S53-S67.
87. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949-54.
88. Fidler IJ. Seed and soil revisited: contribution of the organ microenvironment to cancer metastasis. Surg Oncol Clin N Am 2001;10:257-69.
89. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86:353-64.
90. Folkman J. The role of angiogenesis in tumor growth. Semin Cancer Biol 1992;3:65-71.
91. Fidler IJ, Singh RK, Yoneda J, et al. Critical determinants of neoplastic angiogenesis. Cancer J 2000;6:Suppl 3:S225-S236.
92. Meyerson M, Counter CM, Eaton EN, et al. *hEST2*, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell 1997;90:785-95.
93. Nakamura TM, Morin GB, Chapman KB, et al. Telomerase catalytic subunit homologs from fission yeast and human. Science 1997;277:955-9.
94. Harrigton L, Zhou W, McPhail T, et al. Human telomerase contains evolutionarily conserved catalytic and structural subunits. Genes Dev 1997; 11:3109-15.
95. Bodnar AG, Ouellette M, Frolkis M, et al. Extension of life-span by introduction of telomerase into normal human cells. Science 1998;279: 349-52.
96. Elenbaas B, Spirio L, Koerner F, et al. Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells. Genes Dev 2001;15:50-65.
97. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. Nature 1999;400:464-8.
98. Rich JN, Guo C, McLendon RE, Bigner DD, Wang XF, Counter CM. A genetically tractable model of human glioma formation. Cancer Res 2001;61:3556-60.
99. Lundberg AS, Randell SH, Stewart SA, et al. Immortalization and transformation of primary human airway epithelial cells by gene transfer. Oncogene 2002;21:4577-86.
100. Yu J, Boyapati A, Rundell K. Critical role for SV40 small-t antigen in human cell transformation. Virology 2001;290:192-8.
101. Brookes S, Rowe J, Ruas M, et al. INK4a-deficient human diploid fibroblasts are resistant to RAS-induced senescence. EMBO J 2002;21: 2936-45.
102. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961;25:585-621.
103. Shay JW, Wright WE. Quantitation of the frequency of immortalization of normal human diploid fibroblasts by SV40 large T-antigen. Exp Cell Res 1989;184:109-18.
104. Shay JW, Pereira-Smith OM, Wright WE. A role for both RB and p53 in the regulation of human cellular senescence. Exp Cell Res 1991; 196:33-9.

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105. Macera-Bloch L, Houghton J, Lenahan M, Jha KK, Ozer HL. Termination of lifespan of SV40-transformed human fibroblasts in crisis is due to apoptosis. J Cell Physiol 2002;190:332-44.
106. Stewart N, Bacchetti S. Expression of SV40 large T antigen, but not small t antigen, is required for the induction of chromosomal aberrations in transformed human cells. Virology 1991;180:49-57.
107. Counter CM, Avilion AA, Le Feuvre CE, et al. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J 1992;11:1921-9.
108. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990;345:458-60.
109. Counter CM, Hahn WC, Wei W, et al. Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalization. Proc Natl Acad Sci U S A 1998;95:14723-8.
110. Halvorsen TL, Leibowitz G, Levine F. Telomerase activity is sufficient to allow transformed cells to escape from crisis. Mol Cell Biol 1999; 19:1864-70.
111. Zhu J, Wang H, Bishop JM, Blackburn EH. Telomerase extends the lifespan of virus-transformed human cells without net telomere lengthening. Proc Natl Acad Sci U S A 1999;96:3723-8.
112. Hahn WC, Dessain SK, Brooks MW, et al. Enumeration of the simian virus 40 early region elements necessary for human cell transformation. Mol Biol Cell 2002;22:2111-23. [Erratum, Mol Biol Cell 2002;22:3562.]
113. Zimonjic DB, Brooks MW, Popescu N, Weinberg RA, Hahn WC. Derivation of human tumor cells in vitro without widespread genomic instability. Cancer Res 2001;61:8838-44.
114. Lazarov M, Kubo Y, Cai T, et al. CDK4 coexpression with Ras generates malignant human epidermal tumorigenesis. Nat Med 2002;8:1105-14.
115. Rundell K, Parakati R. The role of the SV40 ST antigen in cell growth promotion and transformation. Semin Cancer Biol 2001;11:5-13.
116. Millward TA, Zolnierowicz S, Hemmings BA. Regulation of protein kinase cascades by protein phosphatase 2A. Trends Biochem Sci 1999;24: 186-91.
117. Colella S, Ohgaki H, Ruediger R, et al. Reduced expression of the Aalpha subunit of protein phosphatase 2A in human gliomas in the absence of mutations in the Aalpha and Abeta subunit genes. Int J Cancer 2001; 93:798-804.
118. Ruediger R, Pham HT, Walter G. Alterations in protein phosphatase 2A subunit interaction in human carcinomas of the lung and colon with mutations in the A beta subunit gene. Oncogene 2001;20:1892-9.
119. *Idem*. Disruption of protein phosphatase 2A subunit interaction in human cancers with mutations in the A alpha subunit gene. Oncogene 2001;20:10-5.
120. Wong AK, Chin L. An inducible melanoma model implicates a role for RAS in tumor maintenance and angiogenesis. Cancer Metastasis Rev 2000;19:121-9.
121. Kerbel RS, Viloria-Petit A, Okada F, Rak J. Establishing a link between oncogenes and tumor angiogenesis. Mol Med 1998;4:286-95.

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CORRECTION

Rules for Making Human Tumor Cells

Rules for Making Human Tumor Cells . In Figure 1 on page 1597, the top box on the right side should have read p14ARF rather than p19ARF; on the left side of the figure, there should have been a barred line (indicating an inhibitory interaction), rather than an arrow, between pRB and E2F.

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