Genomic Components of Carcinogenesis
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Many of the genes encoding growth factors, growth factor receptors, enzymes, and other effector molecules that regulate normal cell growth are designated protooncogenes. Oncogenes, those genes associated with cellular transformation, differ from their protooncogenic progenitors by being mutated, overexpressed, or expressed at inappropriate times or locations in the cell. One of the activities of growth factors is to prime cells to undergo programmed cell death, which is characterized by a series of morphologic changes called apoptosis. In normal cells, specific mediators must be activated or suppressed to bypass programmed cell death. In tumor cells, either the pathways leading to apoptosis are not functional or the mediators that normally "rescue" cells from this fate are overexpressed or constitutively activated. In addition to the biochemical pathways that drive cell division, there are others that limit cell proliferation; these, designated tumor suppressors, anti-oncogenes, or recessive oncoproteins, must be inactivated in normal cells to allow passage through the cell cycle and cell proliferation. In contrast to oncogenes, which are overexpressed or activated in tumors, tumor-suppressor genes are frequently inactivated in tumor cells, either by mutation or deletion. Thus, in normal cells a series of checks and balances must be overcome to allow initiation and continuation of cell division. In tumors, these processes are aberrant, resulting in increased rates of cell division, increases in the proportion of cells in the cell cycle, or increased survival of activated cells. Therefore, tumor cells frequently accumulate genomic alterations, which may result in the activation of a particular array of oncogenes, the inactivation of specific tumor-suppressor genes, and the bypassing of programmed cell death. Trials of antitumor agents that act by exploiting the overexpression of oncogenes in tumors and of the biochemical pathways by which they mediate cell proliferation are currently underway.

Indexing Terms: oncogenes · tumor-suppressor genes · apoptosis · growth factor receptors

The seminal advance in our current understanding of the processes regulating tumor initiation and progression was the observation that genes found in tumorigenic retroviruses, genes from spontaneous human tumors that could transform recipient murine or rat cells, and genes located at sites of chromosomal translocations in human cancer had similar origins (1-5). These genes, commonly called oncogenes, are mutated forms of normal cellular genes designated protooncogenes. These protooncogenes, in turn, are components of the pathways that regulate normal cellular proliferation and differentiation.

Intercellular communication is essential for the processes that determine whether a cell proliferates, enters into a pathway leading to differentiation, or undergoes programmed cell death, or apoptosis. The primary mediators of intercellular communication are polypeptide hormones called growth factors (1-5). Growth factors bind to specific cellular receptors, thereby initiating a limited spectrum of biochemical signals that lead to expression of the genomic programs that result in cellular proliferation, differentiation, or apoptosis. Growth factors can act at a distance as hormones, locally as paracrine factors, through cell–cell contact as juxtacrine factors, on the same cell as autocrine factors, or even intracellularly as intracrine factors. A paradigm established primarily from studies of murine fibroblasts indicates that one family of growth factors renders cells competent to respond to the progression signal generated by a second class of growth factors (1-5). The competence step frequently involves expression of a new array of growth factor receptors. Interaction of growth factors with newly expressed receptors both induces cells to progress through the cell cycle and prevents programmed cell death. Once a normal cell enters the cell cycle, it loses the ability to return to a resting state without completing at least one cycle; in the absence of the progression signal, frequently the cell enters the apoptosis pathway. Coordinated expression of growth factors, their receptors, and activation pathways is required for cellular proliferation and avoidance of programmed cell death. Therefore, one of the potential changes leading to malignant transformation is the ability to proliferate and survive in the absence of this activation cascade.

Oncogenes, the genes responsible for the malignant transformation of cells, act by subverting the normal signaling pathways governing entry into the G1 phase of the cell cycle. The mutation of protooncogenes to oncogenes generally results in gene overexpression, inappropriate gene expression, or expression of an abnormal gene product, either as a result of a single-point mutation or more extensive gene deletions or insertions. These mutations occur such that the resulting "oncoprotein" is freed from the regulatory restraints that usually control the activities of its normal cellular counterpart. As a consequence of these mutations, cells undergo increased rounds of proliferation, eventually leading to malignancy.

In contrast to entry into the G1 phase of the cell cycle, progression through the cell cycle, particularly entry into S phase, requires the coordinated inactivation of a
second series of proteins, the products of genes commonly called tumor suppressors or anti-oncogenes. These growth-inhibitory genes provide a second level of regulation of the cell cycle and cellular differentiation.

Aberrant production or action of growth factors or of the biochemical cascades they initiate acts as a dominant process, requiring changes in only a single allele to lead to oncogenesis. Several genes that regulate programmed cell death had been previously proposed to be oncogenes, which suggests that these genes will be dominant mediators. In contrast, mutations in both copies of a suppressor gene are required to ablate their function; they thus act as recessive oncogenes.

As the processes regulating growth of normal and malignant cells are identified, they become targets for therapy. Indeed, several potential antitumor agents that primarily attack the biochemical pathways induced by growth factors are currently in clinical trials. Similarly, because growth factors and the biochemical pathways they initiate either in their normal protooncogenic or mutated oncogenic forms play a role in tumor initiation and progression, their presence or absence in tumors may correlate with prognosis. Indeed, concentrations of neu and myc, the protein products of protooncogenes neu and myc in ovarian and breast cancer, appear to provide significant prognostic indicators. Thus, the ability to measure the presence and action of these factors could potentially be used as components of screening protocols or to determine which patients may or may not respond to specific therapies.

This article is not planned as an overview of the current literature, but rather utilizes specific oncogenes, tumor-suppressor genes, and anti-apoptosis genes to emphasize particular points. As such, the reader is directed to many of the recent excellent review articles referenced throughout this manuscript for a more detailed description of each gene described.

**Classes of Oncogenes (Table 1)**

The growth and differentiation of eukaryotic cells are subject to tight regulation by both positive- and negative-signaling pathways. All normal quiescent cells can be induced to grow by the action of growth factors (1-4). These growth factors bind to specific transmembrane receptors, which transduce signals across the cell membrane to the cytoplasm. The majority of these growth factor receptors either are protein kinases or activate protein kinases, which phosphorylate substrates in the cell cytoplasm (5-9). Phosphorylation of key regulatory proteins and of second messengers in the cytoplasm activates a chain of biochemical signals, finally leading to the nucleus of the cell and resulting in the activation of various genes responsible for mitogenesis and differentiation (10-12). Similarly, cells are also subject to inhibitory growth signals, which modulate the activation and expression of specific genes and thereby limit the extent of cell proliferation (13-15). Thus, most oncogenes can be classified by their site of action in the signal-transduction pathways and by the protooncogene from which they were derived (16-20). In this review, we will categorize oncogenes as to their potential location in the signal-transduction pathways from the outside of the cell to the nucleus (Figure 1).

**Growth Factors**

The archetypal example of an oncogenic growth factor is platelet-derived growth factor (PDGF), the B chain of...
which is a homolog of the protein encoded by the v-sis gene of the simian sarcoma virus (1–4, 21, 22). Fibroblasts, or any other cell expressing the PDGF receptor when infected by simian sarcoma virus, express large quantities of the v-sis-encoded protein and multiply as a consequence of constant activation of their endogenous PDGF receptors. The v-sis product may bind to and activate the PDGF receptor inside the cell in the endoplasmic reticulum, bypassing the extracellular milieu completely. In contrast to oncogene products located at other levels in the signaling cascade, oncogenic mutations affecting growth factors are rarely found in human cancers. More commonly seen is aberrant production of a growth factor, either in the wrong cell type or at an inappropriate stage of differentiation. For example, the overproduction of wild-type PDGF B (c-sis) dimers is observed in many different tumor lineages (4, 22).

Transforming growth factor α (TGFα), a 50-amino-acid peptide structurally related to epidermal growth factor (EGF), was first identified as a product of retroviral transformed fibroblasts (1–4, 23, 24). The presence of TGFα was subsequently demonstrated in a variety of neoplastic cells and tumors, as well as in embryonic tissues, but was initially not detected in normal adult tissue (25–27). TGFα was believed to be an embryonic growth factor that, when inappropriately expressed in adult tissues, was responsible for neoplasia. TGFα has since been demonstrated to be expressed at very low levels in normal tissues, particularly in skin, where it and the structurally related EGF play an important role in wound healing (23). Therefore, it is not merely TGFα expression, but also its overexpression or expression in inappropriate cell types and resulting autocrine and paracrine effects that lead to cell activation and proliferation, contributing to subsequent development of tumors. Similarly, overexpression of the fibroblast growth factor-like genes int-2 (19, 24, 28) and hst (19, 24, 29) has been detected in tumors of breast, stomach, and various other organs.

Transmembrane Receptors

Tyrosine phosphorylation plays an integral role in the intracellular signaling pathways activated by growth factors. It is not surprising, therefore, that the majority of oncogenes either encode or associate with kinases, particularly tyrosine kinases.

Growth factor receptors are responsible for converting an extracellular growth signal to an intracellular growth signal. The binding of a growth factor to its receptor results in receptor dimerization, receptor autophosphorylation, and tyrosine kinase activation. Phosphorylation of intracellular substrates initiates a cytoplasmic signaling cascade resulting in cell division. Tyrosine kinase activity is essential for the mitogenic response. Conversion of a growth factor receptor tyrosine kinase to an oncogenic phenotype most frequently involves a mutation that causes the receptor tyrosine kinase to be constitutively activated, i.e., in the absence of ligand (5–9, 30).

Mutations affecting receptor tyrosine kinase activity have been extensively characterized in the EGF receptor (5–9, 31, 32). Typical of many receptor tyrosine kinases, the EGF receptor is composed of a large extracellular ligand-binding domain, a single hydrophobic transmembrane domain, and a large cytoplasmic tyrosine kinase domain. Interest in the EGF receptor as a potential oncogenic protein was piqued when the v-erbB oncogene of avian erythroblastosis virus was found to encode a protein with ~95% sequence homology with 400 amino acids of the EGF receptor cytoplasmic domain (31, 32). It was therefore postulated that the v-erbB encoding the avian EGF receptor was the protooncogene of c-erbB, the cellular form of erbB. However, the product of c-erbB lacked the large amino-terminal ligand-binding domain of the EGF receptor, a 34-amino-acid sequence at the carboxyl terminus of the molecule that contained the major sites of autophosphorylation by the EGF receptor and contained multiple point mutations within the kinase domain. The lack of a ligand-binding domain combined with point mutations in the kinase domain probably allows the kinase to be constitutively active in the absence of a growth factor, presumably through a conformational change in the receptor. The tyrosine residues in the carboxyl-terminal tail of the wild-type EGF receptor are believed to block the substrate-binding site of the kinase domain, thus limiting access by exogenous substrates. Ligand activation of the receptor results in autophosphorylation of these sites and an allosteric change, thus permitting phosphorylation of cytoplasmic substrates. Removal of this tail by deleting the appropriate sequence in v-erbB results in a protein that allows constant kinase-substrate association. Therefore, the v-erbB product constantly signals the cell to divide as a consequence of bypassing the requirement for growth factor and by removing a negative regulatory component of the EGF receptor. The oncogenic forms of the cystostatic factor CSF-1 receptor, a product of v-fms (33), and the stem cell factor receptor, a product of v-kit (34), both possess truncations of the carboxyl-terminal tail of their kinase domains. As seen in v-erbB, v-kit also lacks a large portion of its extracellular ligand-binding domain (34).

Whereas the mutations observed in these viral oncogenes are severe, much smaller mutations in cellular genes have produced oncogenic receptor tyrosine kinases. The neu oncogene is generated from its protooncogene by a single point mutation such that a glutamic acid residue is substituted for a valine residue in the transmembrane domain of the receptor (5–9, 35, 36). This substitution results in the constitutive dimerization and thus activation of receptor tyrosine kinase, which is essential for cell transformation. Similarly, a point mutation in the extracellular ligand-binding do-

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8 Nonstandard abbreviations: PDGF, platelet-derived growth factor; TGF, transforming growth factor; EGF, epidermal growth factor; SH2, SH3, src homology regions 2 and 3; FPKC, protein kinase C; GAP, GTPase-activating protein; and RB, retinoblastoma gene.
main of the v-fms product contributes to its oncogenic potential (37).

Far more common than mutation is the amplification of genes encoding receptor tyrosine kinases and the overexpression of these receptors, particularly in conjunction with overexpression of their respective growth factors. Members of the EGF receptor family are the most commonly overexpressed receptor tyrosine kinases in human cancers. The EGF receptor gene is amplified or overexpressed in a variety of tumor types, including squamous cell carcinoma, glioblastomas, and adenocarcinoma of the breast and ovary. Concurrent expression of the EGF receptor ligand family, of which five members have been cloned, is also commonly observed in tumors (8, 38). Overexpression of the protooncogenic neu-encoded receptor tyrosine kinase primarily as a consequence of gene amplification has been observed in −30% of both breast (39) and ovarian (40–43) carcinomas. Whether neu overexpression alters the prognosis of breast cancer patients remains controversial; however, neu overexpression in ovarian cancer cells correlates with a poor prognosis. Thus overexpression of the neu-encoded receptor may play a crucial role in the initiation or progression of these diseases.

Cytoplasmic Signaling Molecules

A complex network of signaling molecules acts within the cell cytoplasm. The action and interaction of these molecules are essential for the transduction of extracellular messages from the cell membrane to the nucleus. Mutations of cytoplasmic signaling molecules, in particular kinases and G-proteins, can result in cell proliferation in the absence of extracellular signals.

Nonreceptor tyrosine kinases. The src family of tyrosine kinases are not inserted directly through the cell membrane like receptor tyrosine kinases, but rather associate with the cell membrane through a myristylated amino terminus. Members of the src family of kinases include blk, fgr, fyn, hck, icc, lyn, and yes (44). Although each of these kinases is distinct with respect to sequence and substrate specificity, members of the src family share highly conserved noncatalytic sequences known as src homology regions 2 and 3 (SH2 and SH3) (44–47). SH2 domains have been demonstrated to bind to phosphorylated tyrosine residues, thus providing a mechanism for the formation of stable complexes between the src kinases and other signaling molecules, including receptors. The SH2 motif is found in many of the cytoplasmic signaling molecules, kinases and others, apparently for a similar purpose. The function of the SH3 domain is less clearly defined, although recent evidence points to a role in the association of specific proteins with the cytoskeleton and has been linked to association with small G-proteins (45–47).

Src was first identified as a 60-kDa product of the Rous sarcoma virus, v-src, isolated from an avian tumor (44). Unlike c-src, v-src expresses a carboxyl-terminal deletion of an important negative-regulatory site, tyrosine 527. Phosphorylation of tyrosine 527 negatively regulates src kinase activity, presumably as a result of interaction between this phosphotyrosine residue and the SH2 domain of src itself, which lies upstream of the kinase domain, thereby blocking any interaction between the src kinase domain and exogenous substrates. Dephosphorylation or mutation of this site results in src activation. Membrane association of src through myristylation of the amino-terminal glycine also appears to be an important event that positively regulates kinase activity and is clearly required for its transforming activity (16, 19, 44).

The overexpression of c-src is not sufficient to cause transformation. Mutations affecting the carboxyl-terminal tail of the kinase domain as well as myristylation are required for full transformation to occur. Therefore, possible mutations of the src gene, resulting in its conversion to an oncogene, include point mutations affecting the carboxyl-terminal autophosphorylation site or increased dephosphorylation of tyrosine 527 by increased action of a phosphatase. Alternatively, mutations affecting the src SH2 domain could decrease interaction with phosphotyrosine 527 and possibly alter its association with other SH2-containing signaling proteins (16). Indeed, mutation of the src SH2 domain leads to an increase in association with the second messenger, phosphatidylinositol-3-kinase. In addition, activation of the PDGF receptor leads to association with src, thus directly linking src activity with receptor activation (48). Alternatively, activation of src may be achieved as a consequence of the overexpression of other SH2-containing molecules. These may compete with src for phosphotyrosine 527, thereby freeing the kinase domain to interact with other substrates (16). Cells transformed with the viral oncogene, v-crk, which consists of little more than an SH2 and SH3 domain fused to a viral gag sequence, demonstrate increases in tyrosine phosphorylation. V-crk, in fact, has been demonstrated to associate with p60 c-src and is presumed to activate src by interaction with specific phosphotyrosine residues (16, 45–47, 49).

An increase in src kinase activity has been noted in nearly 100% of colon carcinomas and in both benign and malignant polyps, which are precursors to colon cancer (50). Src activation, therefore, may represent an event in the multistep pathway leading to colon cancer.

C-abl is the cellular counterpart to v-abl, encoded by the Abelson murine leukemia virus, and represents another family of nonreceptor tyrosine kinases (6, 7). C-abl is normally a weakly activated tyrosine kinase targeted to the nucleus, where it subserves its function. Activation of c-abl through amino-terminal fusion with either the myristylated gag sequence, as found in v-abl, or with the amino-terminal domain of the GTPase-activating protein, bcr, by chromosomal translocation can contribute to malignant transformation. The characteristic Philadelphia chromosome observed in ~90% of chronic myelogenous leukemia patients and to a lesser extent in acute lymphocytic leukemia patients is the consequence of a reciprocal chromosomal translocation of abl on chromosome 9 to the bcr locus on chromosome 22 (16, 51–53). Curiously, fusion with either the gag or the bcr
sequence leads to the relocation of at least some abl from the nucleus to the cytoplasm. This change in milieu may contribute in part to cellular transformation by allowing phosphorylation of inappropriate substrates involved in signaling pathways (16, 54). Increases in abl activity, in conjunction with the deletion of amino-terminal sequences as a consequence of translocation, indicate that the regulatory sites of abl are located near the amino terminus of the kinase domain (55–57).

Serine/threonine kinases. Although serine/threonine kinases play important roles in signal transduction, particularly as second messengers, relatively few serine/threonine kinase oncogenes have been observed. In general, serine/threonine kinases tend to operate farther downstream as second messengers in the signaling process, acting to integrate signals originating at the cell membrane with those operating at the nucleus (2, 6, 7, 16, 30, 58).

Activation of the raf family of serine/threonine protein kinases has been identified in human stomach cancer and in cell lines (2, 16, 59). Many growth factor receptors have been demonstrated to increase raf activity, which is positively controlled by phosphorylation of serine and tyrosine residues. Raf kinases act primarily as second messengers, and more specifically are thought to act downstream of ras, because oncogenic raf can release cells from growth arrest induced as a result of ras blockade. Studies by several groups have demonstrated that raf can be converted to an oncogene by truncation, amino-terminal fusion, and site-specific mutations. Overexpression of raf in cell lines induces an increase in DNA synthesis and transcriptional activation (2, 16, 59), perhaps contributing to oncogenic transformation.

The serine/threonine kinase c-mos has been identified as a component of cytotstatic factor, which is involved in the regulation of the cell cycle, in particular the stimulation and arrest of M phase (6, 7, 30, 60, 61). An increase in mos expression induces the activation of the maturation-promoting factor, composed of cyclin B and cdc2 kinase. Selective phosphorylation and dephosphorylation of maturation-promoting factor is required for the onset of M phase, and cdc2 kinase has been demonstrated to be a substrate for mos (60, 61). V-mos was initially isolated as a product of the Moloney murine sarcoma virus; mutations of c-mos in human tumors have not yet been observed. Overexpression of c-mos in NIH3T3 cells is sufficient to cause transformation, so it is likely that mos induces its oncogenic effects by overexpression (60, 61).

Although mutations in the important second messenger protein kinase C (PKC) have not been identified in vivo, it is the target for activation by the phorbol ester tumor promoters. These tumor promoters activate PKC in a fashion similar to that of its physiological activator, diacylglycerol, which is generated in response to receptor-induced turnover of membrane phospholipids (62, 63).

G-proteins. Guanyl nucleotide-binding proteins (G-proteins) can be divided into two large groups based on size, number of members in the complex, or intrinsic GTPase activity. The family of large G-proteins consists of heterotrimeric with significant endogenous GTPase activity. In contrast, members of the family of small G-proteins are monomeric proteins with limited endogenous GTPase activity; however, their GTPase activity can be modified by exogenous proteins or GTPase-activating proteins.

1. Heterotrimeric or large G-proteins. Large G-proteins are heterotrimeric proteins composed of plasma membrane-associated α, β, and γ subunits that link receptor activation with effector proteins such as adenyl cyclase, subsequently activating the cAMP-dependent kinase signaling cascade (19, 64–67). In the inactive state, the heterotrimer remains closely associated with its receptor. The α subunit, which contains endogenous GTPase activity, binds GDP and is inactive. After ligand–receptor association, the α subunit releases GDP and binds GTP. The active α subunit then dissociates from the βγ subunits and associates with and activates adenyl cyclase, increasing cAMP concentrations. Because of the endogenous GTPase activity of the α subunit, GTP is hydrolyzed to GDP, and the α subunit dissociates from adenyl cyclase and reassociates with the βγ subunits. A wide variety of α subunits have been identified, with different specificities and localization. Mutations that generate GTPase-deficient mutants of G-protein α subunit have been found in many human pituitary tumors (19, 68). Presumably, this occurs because a defect in GTPase activity would result in a prolonged association of adenyl cyclase or other effector molecules with the α subunit and therefore prolong activation of effector pathways, including adenyl cyclase, and increase cAMP production. High concentrations of cAMP are mitogenic in growth factor-secreting pituitary tumors (68). Point mutations in the α subunit are also detected in endocrine tumors of the adrenal cortex and ovary (69).

2. Monomeric or small G-proteins. Ras and its related family members are the best-characterized members of the second subset of guanine nucleotide-binding proteins, which are important regulators of mitogenic signals (70–73). Ras itself is believed to work downstream of the majority, if not all, of the membrane-associated tyrosine kinases but upstream of raf, as previously described. There are three similar oncogenic ras genes: N-ras, found in a human neuroblastoma; Ki-ras, encoded by the Kirsten sarcoma virus; and Ha-ras, encoded by the Harvey sarcoma virus (19, 73). All three genes encode a 21-kDa protein that is anchored to the cell membrane by a myristic acid at a cysteine residue four amino acids from the amino terminus. As with the src family, association with the plasma membrane is essential for transformation.

Activating mutations of ras are frequently detected in a wide range of human cancers and as such have become the subject of intense investigation. In particular, the mutation of codons 12 and 13 or the mutation of 59 or 61—or as in the virally encoded ras, both—activates ras as an oncogene. Ras overexpression is less frequently observed. Ras mutations are detected in 30–50% of lung
and colon carcinomas (19, 74), and in >90% of pancreatic tumors (19, 74). That oncogenic mutations in ras can be detected in the stool of patients with bowel tumors indicates a possible mechanism for screening for these tumors in the future.

Characteristic of G-proteins, p21 ras interacts with effector proteins when present in the activated GTP-bound form and dissociates from them after hydrolysis of GTP to GDP. In contrast to the α subunits of heterotrimeric G-proteins, p21 ras possesses a very low amount of intrinsic GTPase activity. A 120-kDa protein known as ras GTPase-activating protein (ras-GAP) has been identified (75, 76) that is believed to act as a complex with ras. Ras-GAP acts to negatively regulate ras activity by stimulating hydrolysis of GTP and thus terminate ras activity (75, 76). GAP also contains a SH2 domain, which may provide a link between ras, tyrosine kinases, and their substrates (17, 44, 45). Transforming ras proteins often contain point mutations that inactivate their intrinsic GTPase activity, such that ras is present in the constitutively active GTP-bound form (73, 77).

Nuclear Proteins

The end point of the growth factor-mediated signal cascade ultimately lies in the nucleus, where mitogenic signals activate the transcription of genes whose products are required for cell division and differentiation. Gene activation is mediated by a large variety of transcription factors that act as heterodimeric or homodimeric DNA-binding proteins and stimulate or inhibit gene transcription (11, 12). Not surprisingly, from the studies described above, the action of many transcription factors appears to be controlled, at least in part, by phosphorylation (10).

Of the transcription factors involved in the promotion of cell-cycle progression, several have been identified as viral oncogenes. These include myc, fos, jun, myb, rel, and ets (10–12, 19). Mutations in transcription factors that cause positive activation of genes required for cell growth, or alternatively those that cause a loss of negative regulatory function, can result in the inappropriate gene transcription (82). Typical alterations found in transcription factors include overexpression and gene amplification as well as deletions, insertions, and point mutations that eliminate negative regulatory domains of the transcription factors or diminish their association with other proteins that may negatively regulate their activities.

In all cases of Burkitt lymphoma, whether of the endemic African form or of sporadic origin, myc is overexpressed as a result of reciprocal chromosomal translocation from chromosome 8 to the immunoglobulin loci on chromosomes 2, 14, or 22 (53, 83, 84). Overexpression of myc as a result of gene amplification has been documented in a variety of tumors, including stomach, colon, lung, brain, ovary, and several leukemias. The degree of amplification correlates with the stage of cancer, in particular with increases in the aggressiveness of the tumor and thus poor prognosis.

The transcription factors fos and jun operate as dimers, either as fos–jun dimers or jun–jun homodimers, and bind to specific DNA serum response elements or to phorbol ester response elements (10–12, 19, 85). Both amino- and carboxyl-terminal deletions as well as point mutations have been observed in oncogenic forms of both fos and jun. In fact, to activate transcription, jun need only retain a proline-rich activation domain, a basic DNA-binding domain, and a leucine zipper domain required for dimerization.

The oncogenic protein v-erbA is derived from the thyroid hormone receptor (86). C-erbA resides in the nucleus and is capable of binding to specific DNA sequences. The binding of its ligand, triiodothyronine, results in transcriptional activation. Compared with its protooncogenic form v-erbA contains both amino- and carboxyl-terminal deletions as well as several substitutions, which may affect its binding of ligand. Therefore, like the EGF receptor-derived oncogenic protein v-erbB, v-erbA is active independently of the presence of exogenous ligand.

Anti-Apoptosis Genes: Bcl2

In an alternative mechanism that may contribute to oncogenesis, expression of a gene promotes an increase in cell number not by promoting cell growth but rather by preventing programmed cell death. The bcl2 oncogene was initially discovered as a result of studies of the commonly observed point-break mutation between chromosomes 14 and 18, found in a high proportion of human follicular B-cell lymphomas (78–80). B cells affected with this mutation show no apparent increase in the rate of cell proliferation, but rather have extended survival times and do not undergo programmed cell death.

Characterization of the bcl2 gene yielded the identification of two gene products, bcl2α and bcl2β, which differ only by the presence of a postulated transmembrane domain in the more commonly found α form (78). No differences in sequence have been determined between the translocated bcl2 and its naturally occurring counterpart. Therefore, the inappropriate expression or overexpression of bcl2 as a consequence of its translocation from chromosome 18 to a position adjacent to the immunoglobulin heavy-chain locus on chromosome 14 is probably responsible for its oncogenic properties. Bcl2 has been localized to the mitochondrion (81), where it clearly plays a role in inhibiting apoptosis. The expression of bcl2 appears to be restricted to cells that are subject to regulation by apoptosis, especially hematopoietic cells. Transfection of bcl2 into certain cell types enhances the survival of these cells in the absence of exogenous growth factors, similar to observations in human follicular B-cell lymphoma.

Tumor-Suppressor Genes

Oncogenes have been extensively studied and characterized for years, but only within the past decade have the genes responsible for the negative growth regulation of cells, or tumor-suppressor genes, been identified.
Mutations of p53 are the most common defect detected in human cancers. Mutations affecting the activity of the p53 protein have been extensively characterized and mapped to specific "hot spots" (87). Germ-line mutations of p53 have been identified in Li–Frauneni syndrome, in which affected family members are susceptible to a wide variety of tumors early in life (88). Nevertheless, in the Li–Frauneni syndrome, an increase in tumors of a specific group of cell lineages is observed rather than an increase in the occurrence of all types of tumors. Thus, inactivation of p53 contributes to the development of specific types of tumors. This was originally thought to reflect a limited role for p53 in growth regulation; however, more recent studies with mice that lack functional p53 through homologous recombination indicate that the occurrence of specific tumors in Li–Frauneni patients may be due to a permissive role of p53 mutations in the development of tumors, as a result of the genetic or environmental background of the patients. This is based on the observation that mice with the p53 gene deleted do not express the same patterns of tumors that are seen in Li–Frauneni patients, nor do they exhibit a generalized increase in tumors; rather, these mice exhibit an earlier onset of tumors commonly seen in normal mice of the same strain.

In nonfamilial cancers, particularly colon cancers, mutation of p53 has been determined to be an important event in the chain of mutations leading to carcinogenesis (89, 90). In addition to mutations of p53 itself, the overexpression of a p53-binding protein, MDM2, has been observed in many human sarcomas (87, 91). The amplification of this gene and its protein is believed to interfere with the activity of normal p53 protein.

The actual role of p53 in the cell is not yet clear, but recent research strongly implicates p53 in transcriptional control (87, 92). Structurally, p53 possesses an acidic amino-terminal domain, commonly found in many already characterized transcription factors. p53 has been demonstrated to bind to specific DNA sequences both in vivo and in vitro and, in fact, has been shown to activate transcription of reporter genes from plasmids that encode upstream p53-binding sites. It appears, then, that p53 activates the transcription of growth-inhibitory genes rather than inhibiting the transcription of growth-promoting genes. Commonly found mutations in p53 destroy the ability of the protein to bind to p53-specific sequences and lose their ability to activate reporter genes (87). p53 is believed to act as a tetramer, and mutations affecting its oligomerization have also been identified.

**RB**

The product of the RB gene was first identified through investigation of the familial form of this disease (13–15). Children in affected families usually present between the ages of 1 and 3 years with multiple bilateral tumors of the retina. Individuals who develop retinoblastoma sporadically do so much later in life, most frequently with one tumor in a single eye. Deletions of the long arm of chromosome 13 were commonly ob-
served in affected individuals. Knudson, at the time, postulated that children with familial retinoblastoma were born with a germ-line mutation in a specific gene that negatively regulated the growth of cells; a second "hit" to the remaining normal allele resulted in the deregulation of normal cell growth and the formation of tumors (93).

Knudson's hypothesis was confirmed when RB was cloned and localized to chromosome 13 (94). RB encodes a 110-kDa nuclear phosphoprotein, which localizes in the nucleus and associates with DNA. Expression of the RB protein has been observed in a wide variety of tissues; however, it is inactivated in only a limited number of tumors.

Similar to p53, wild-type RB seems to play a role in regulating progression through the G1 checkpoint of the cell cycle. RB exists in "hyper-" and "hypo-" phosphorylated forms during different stages of the cell cycle (95, 96). In growth-arrested cells and those cells in the G1 phase of the cell cycle, RB is hypophosphorylated. Just before entry into S phase and throughout the G2/M phases of the cell cycle, RB undergoes extensive phosphorylation of threonine and serine residues. Dephosphorylation occurs before reentry into G1 and at growth arrest. Therefore, the hypophosphorylated form of RB is believed to be responsible for the negative regulation of cell-cycle progression. How RB accomplishes this has yet to be determined. The association of RB with known transcription factors has been observed, as has its association with DNA. RB forms complexes with virus-encoded proteins, such as E1A and large T antigen encoded by the SV40 virus and E7 encoded by the human papilloma virus. These viral proteins bind specifically to the hypophosphorylated form of RB, therefore targeting the active form of RB for inactivation (96).

Thus the RB and p53 proteins appear to function primarily at a checkpoint that regulates progression from the G1 phase of the cell cycle to S phase. The RB and p53 genes are activated or expressed at greater levels in cells that have undergone genetic damage or that lack nutrients required for successful and correct DNA replication. Thus these tumor-suppressor genes prevent cells susceptible to DNA mutations from passing through S phase. Cells lacking tumor-suppressor function lack this "checking" process and are thus susceptible to genetic mutation, perhaps resulting in activation of oncogenes or inactivation of tumor-suppressor genes.

Clinical Applications of Oncogene Research

The presence of oncogenes or loss of tumor-suppressor genes could potentially be used for screening patients and to facilitate early diagnosis, establish prognosis, tailor therapy to the individual, or assess response to therapy. Currently, studies of RB and p53, as well as of several less-well-characterized potential tumor-suppressor genes, are used in counseling patients and establishing risk for tumor development. In addition, with a better understanding of mediators of oncogenesis, new, more rational therapies that will specifically target cancer cells and oncogenes are within the realm of possibility. Most of these approaches are currently at the experimental or evaluation phase; however, with our rapid increase in understanding of the mechanisms involved, it appears likely that evaluation of oncogenes and tumor-suppressor genes will soon play a routine role in patient management.

One application of this research is the use of oncogenes as markers for tumor progression and prognosis. Increased expression of the EGF receptor and the product of the closely related neu protooncogene has been correlated to a poor prognosis in a variety of cancers (39-43, 97, 98). For example, >30% of ovarian carcinomas show an increase in neu protein expression, and individuals with neu-positive tumors have decreased survival times—15 months vs 33 months in those whose tumors express normal levels of neu (39-42, 96). Thus, determination of expression of neu-encoded receptors could be used to tailor treatment for specific patients.

If an oncogene is overexpressed in tumor cells, it may be possible to target specific chemotherapeutic and radiotherapy to affected cells, provided the targeted signaling molecule is not widely expressed in normal, nonmalignant cells. Furthermore, if the protooncogene is overexpressed in tumor cells, particularly as the result of gene amplification as is observed with both neu and the EGF receptor gene, targeted therapy could have a significant therapeutic ratio. Strikingly, the EGF receptor and neu genes are frequently coexpressed on tumor cells, a circumstance that happens rarely, if at all, in normal tissue. Thus, targeting therapy to cells that coexpress these protooncogenes could result in an effective, relatively nontoxic therapy. Much work is being done to identify mechanisms to target therapy to specific surface markers on tumor cells. Two major approaches have entered clinical trials. The first is based on utilizing cell-surface markers to target antibodies to cells. These antibodies could be radiolabeled or conjugated to toxins to increase their ability to kill tumor cells (99-101).

The second approach is based on the signaling pathways utilized by oncogenes and protooncogenes. Because so many of the oncogenes either encode or activate tyrosine kinases, inhibitors of tyrosine kinase activity may provide a useful mechanism by which to inhibit the growth of malignant cells (102, 103). Again, such techniques must take advantage of the overexpression or activity of certain tyrosine kinases in some malignancies. The tyrphostins, inhibitors of tyrosine kinases, selectively inactivate different kinases involved in cell signaling by acting as specific pseudosubstrates or "suicide inhibitors." Various tyrphostins have been demonstrated to inhibit the action of one receptor tyrosine kinase over three orders of magnitude more effectively than other receptor tyrosine kinases. Tyrphostins that inactivate the EGF receptor are effective inhibitors of the growth of EGF receptor-expressing tumors in animal model systems and are currently in trials for the treatment of human diseases.

Some oncogenes depend on association with the cell
membrane for their activity. This implicates the process of fatty acylation by farnesyl and myristyl groups, such as is required by the ras and src oncogenes, respectively, as a target for therapy. Drugs that inhibit the fatty acylation have been developed and show anti-oncogenic potential (104).

Synthetic oligodeoxynucleotides complementary to specific mRNA sequences have been widely used in the last decade to inhibit the production of a large variety of proteins, including oncogenes in vitro (105). Although the mechanism of “antisense oligo” action is not clearly understood, the binding of antisense sequences to specific RNAs results in decreased protein synthesis, either by inhibiting translation of mRNA or by targeting the mRNA–antisense RNA complex for degradation by ribonucleases. The use of antisense oligonucleotides in vivo is limited by the instability of the oligonucleotides and the inability to target them appropriately. Preliminary in vivo animal studies have demonstrated success in inhibiting tumor growth by local perfusion or intraperitoneal injection of antisense oligonucleotides directed at genes involved in transformation.

Perhaps the area that would be the most significant for patient care would be the use of our rapidly evolving understanding of oncogenes or tumor-suppressor genes as a mechanism of identifying either those patients at increased risk for cancer or for screening for the presence of tumors at an early treatable stage. Indeed, patients with familial abnormalities in p53 and RB as well as several other tumor-suppressor genes can now be identified. By studying either the tumor-suppressor genes or linked loci, we can now test individuals from pedigrees with increased incidence of specific familial cancers and determine whether or not they express the aberrant gene. Thus, the members of a family with normal tumor-suppressor genes who have greatly increased risk of developing tumors can be identified and separated from those family members who have the same level of risk as the general population. This could be utilized to trigger increased surveillance, screening for tumor presence, or even prophylactic therapy. It may even eventually be feasible to screen antenatally for the presence of specific abnormal tumor-suppressor genes. Some studies are currently being aimed at the possibility of correcting the defect in specific genes, either antenatally or postnatally through gene therapy.

Population screening with oncogenes is not yet practical; however, recent studies have shown that abnormal ras can be detected in the stool of a significant number of individuals with bowel cancers (106). Furthermore, some individuals with tumors exhibit antibodies against specific oncogenes and tumor-suppressor genes such as p53. Similarly, the presence of shed oncogenes or protooncogenes such as neu in serum could be part of a screening test for specific types of cancer. Although none of these markers is as yet sufficient to serve as a component of tumor-screening plans, it is far too early for pessimism to enter into the field. With our increased understanding of the genetic defects in tumors and the increased ability to detect them, either through application of new molecular biology techniques or more classic studies, practical screening protocols for oncogenes or tumor-suppressor genes will surely be developed.

In summary, identification and characterization of oncogenes and tumor-suppressor genes have allowed dissection of the growth-signaling pathways that participate in the proliferation of both benign and malignant cells. This comprehension of the mechanisms of tumorigenesis may lead to advances in patient management, perhaps including population screening, patient counseling, and therapeutic intervention. Such studies may eventually permit the design of pharmacological agents that can block uncontrolled cell growth at the site of the defect and lead to a more rational and effective treatment for cancer.

References


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