Malignancy: An Evolving Definition of a Cancer Cell

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ABBREVIATIONS: Cdk = cyclin-dependent kinases; PTK = protein-tyrosine kinases.

INDEX TERMS: malignancy; tumor.

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Focus Continuing Education Credit: *see pages 60 to 63 for learning objectives, test questions, and application form.*

LEARNING OBJECTIVES

1. Compare and contrast the functional characteristics of oncogenes and tumor suppressor genes.
2. Summarize the four broad functional categories of oncogenes.
3. Define and explain the role of cyclins and Cdks in regulating cell cycle progression.
4. Describe the 'cell-cycle checkpoints'.
5. Explain how loss of Rb protein can contribute to oncogenesis.
6. Summarize the roles of caspases, the Bcl-2 family of proteins, and IAPs (inhibitors of apoptosis) in the regulation of apoptosis.
7. Correlate overexpression of Bcl-2 and Bcl-X_L, or loss of function of p16 or p27, with oncogenesis.
8. Describe the role of telomerase in tumorigenesis.

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In recent years there has been an explosive growth in our understanding of cancer cell biology. For most of the 20th century, cancer was assumed to be a disease of uncontrolled proliferation of cells. A (malignant) neoplasm was defined as 'an abnormal mass of cells typically exhibiting uncontrolled and progressive growth'.[1] A more current definition of malignancy recognizes the complex interplay of an imbalance between cell proliferation, differentiation, and cell death.[2] The molecular mechanisms regulating cell proliferation and cell death (apoptosis) have been among the areas of biomedical research generating intense interest for the past 20 years. As these molecular controls have been defined and refined, our understanding of the molecular basis of malignancy has similarly been advanced, and new diagnostic and therapeutic approaches for various human malignancies are being developed.

ONCOGENES AND TUMOR SUPPRESSOR GENES

Cancer is a genetic disease. The characteristics that make a cell malignant are stably inherited during the process of cell division. We also recognize that most tumors are clonally derived from a single common progenitor cancer cell that typically divides to generate a tumor of identical sibling cells. Together, these two observations indicate that the disease phenotype is determined by the tumor cell DNA.

A major advancement in understanding cancer cell biology was the discovery that certain viruses, when inoculated into animals, had the capacity to cause the development of tumors. This critically important observation resulted in a massive investigation to identify the particular viral genes capable of inducing malignant transformation. The tumor-causing viruses were shown to carry discrete genetic elements, oncogenes, which are responsible for their ability to transform cells. The proteins encoded by these viral oncogenes influence various steps of the cell cycle (cell proliferation), such as initiation of DNA replication and the transcriptional control of genes. Of even greater interest was the finding that many viral oncogenes have counterparts in the normal human genome, now called cellular proto-oncogenes. Proto-oncogenes are highly conserved genes, which play key roles in cellular metabolism and growth. These proto-oncogenes, which regulate normal cellular processes, can induce malignant growth, i.e., become an 'oncogene' if the normal controls regulating their expression are removed. The identification of cellular proto-oncogenes verified that our genomes carry genes with the potential to alter cell growth patterns dramatically, and even to kill us.

In all cases where the functions of oncogenes are known, the proteins encoded by them are components of the signaling pathways

by which cells receive and execute growth instructions. The mutations that convert these proto-oncogenes to active oncogenes are typically either structural mutations resulting in the constitutive activity of a protein without an incoming signal, or regulatory mutations that lead to the production of the protein at the wrong time or place. In either case, the result is a persistent internal growth signal in the absence of any external stimuli.

Oncogenes encode a diverse group of proteins, involved in many aspects of cellular growth and metabolism (Table 1):

1. Some encode growth factors, the molecules that are themselves the signals for cell proliferation, resulting in an 'autocrine' growth stimulation.
2. Other oncogenes encode (altered) growth factor receptors, capable of triggering growth-promoting signals even in the absence of growth factor ligand.
3. The largest class of oncogenes encodes proteins that associate with growth factor receptors inside the plasma membrane, which function as signal transducers, passing signals from the growth factor receptor to downstream targets. Many of these molecules are protein-tyrosine kinases (PTK) found on the inner surface of the membrane. Tyrosine phosphorylation and dephosphorylation are recognized as one of the principal signaling mechanisms in mammalian cells.[3] Often, the products of these oncogenes are signaling molecules that exist in a constantly activated state, in the absence of growth factor/receptor interaction and signaling.
4. Some oncogenes are transcription factors, proteins that directly regulate gene expression. These proteins bind DNA and function to control the expression of cellular genes required for proliferation or cell death. As above, the oncogenic version of these genes represent constitutively activated proteins, which exert their regulatory influences on gene transcription in the absence of growth factor, growth factor receptor, or signal transducer molecule instructions to do so.

The activation of a proto-oncogene, resulting in the creation of an oncogene, generally occurs via one of two processes. The first is a mutation that alters the protein product of the proto-oncogene, resulting in a qualitative change in structure and function. The second mechanism is to alter the regulation of proto-oncogene expression so that a quantitative effect on function occurs, resulting in the overproduction of an otherwise normal oncogene product.[4]

Oncogene mutations tend to be activating mutations, which functionally are dominant to wild type gene products, i.e., they produce proliferation signals even when a single copy of the oncogene is present. The human genome also contains genes that function to preserve a normal pattern of growth, i.e., they function as growth-suppressing genes, acting to prevent tumorigenesis. Originally called anti-oncogenes, these genes are now known as tumor suppressor genes, because of their ability to inhibit cell division or to regulate cell cycle progression. Mutations of tumor suppressor genes that result in a loss of function of the gene would potentially lead to a loss of growth control. However, since the remaining wild type allele would continue to function, mutations of tumor suppressor genes are functionally recessive at the cellular level (Table 2).

It is possible that any gene that plays a key role in cellular growth can become an oncogene, if mutated in an appropriate way. Although 'cancer' is a collection of hundreds of distinct disease processes, a unifying feature is that all types of cancer result from uncontrolled, excessive, or improperly regulated cell growth.

Table 1. Functional definitions of oncogenes

Growth factors
Growth factor receptors
Signal transducer molecules
Transcription factors

CELLULAR PROLIFERATION: THE CELL CYCLE

Cell division is a fundamental process required throughout the life of all eukaryotes. The biochemical and morphological stages that a cell passes through when stimulated to divide are referred to as the cell cycle, which is conveniently divided into several phases: G_1 (Gap-1), S (DNA synthesis), G_2 (Gap-2), and M (mitosis) (Figure 1). Not all of the cells in the body are actively dividing, i.e., are actively engaged in the cell cycle. Cells may exit the cell cycle at G_1 and enter a nonproliferative phase called G_0 or quiescence. In response to specific mitogenic stimuli, quiescent cells will exit G_0 and reenter the cell cycle at the level of early G_1. In unicellular organisms such as bacteria, cell division is dependent only on an adequate supply of nutrients. In mammalian cells, all cell division cycles are initiated by specific growth factors or mitogens that drive the cell from $G_0 \rightarrow G_1$.

Table 2. Properties of oncogenes and tumor suppressor genes

Property	Oncogenes	Tumor suppressor genes
Nature of mutation	Dominant Gain of function	Recessive Loss of function
Inherited mutant allele	Never observed	Common – basis for inherited predisposition in cancer

Figure 1. Cellular proliferation

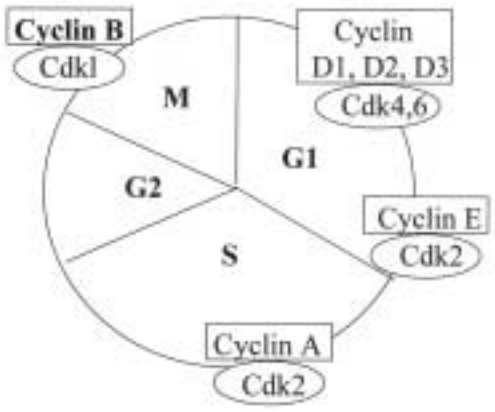


Table 3. Cell cycle regulatory proteins

Cell-Cycle	Cyclin	Partner Cdk
G_1	D1, D2, D3	Cdk4, Cdk6
G_1/S	E	Cdk2
S	A	Cdk2
M	A	Cdk1 (Cdc2)
	B	Cdk1 (Cdc2)

G_1 is the period of cell growth where the synthesis of components necessary for replication takes place. As cells transit through G_1, they pass through the 'restriction point' or 'R'. R occurs in late G_1 and is the point in the cell cycle after which the cell is no longer responsive to extracellular signals. In other words, the cell is committed to completing that cell cycle *independent* of mitogenic stimuli, i.e., cell cycle completion becomes autonomous.[5] As cells pass this restriction point, they traverse the G_1/S boundary and enter the S phase of the cycle where DNA synthesis occurs, followed by the G_2 phase, and finally mitosis where nuclear division (karyokinesis) and cytoplasmic separation (cytokinesis) occur.

The actual mechanisms involved in the molecular regulation of cell cycle progression have been the focus of significant research efforts for the past decade.[6] The fundamental task of the cell cycle is to *faithfully* replicate DNA once during S phase and to distribute *identical* chromosome copies *equally* to both daughter cells during M phase. Organized progression through the cell cycle ensures that this normally takes place. Cells must make certain that chromosome duplication and segregation occur in the correct order, i.e., S → M → S → M. They must also see that the next event in the cycle only begins when the previous events have been successfully completed. Entry into and exit from each phase of the cell cycle are normally tightly regulated.

CYCLINS AND CDKS

Movement through the cell cycle is regulated by enzymatic activities of specific and unique kinases. These kinase proteins, Cdks or cyclin-dependent kinases, phosphorylate target molecules important in controlling cell-cycle progression. To be active, the kinase (Cdk) must be complexed with a regulatory subunit named cyclin, hence the name, cyclin-dependent kinase. Numerous cyclins and Cdks exist in the cell. Different kinase complexes, with differing cyclin, and Cdk components, drive the cell from one stage of the cell cycle to the next. The sequential activation of successive cyclin/kinase complexes, each of which in turn phosphorylates key substrates, facilitates or regulates the movement of the cell through the cycle (Figure 1). The concentration of the different cyclin proteins rises and falls at specific times during the cell cycle; hence they are 'cycling' proteins. In contrast, protein levels of the kinase subunit remain constant throughout the cell cycle. Different cyclin/Cdk complexes are functional at different phases of the cell cycle (Table 3).

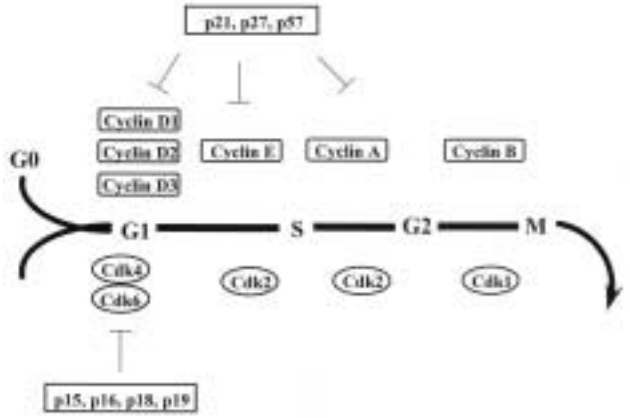
As discussed above, a mammalian cell must receive external signals, growth factors, and/or hormones, which trigger the cell to initiate cell cycle progression.[7] These external signals result in an increase of one or more of the D cyclins, which complex with their partner Cdks (Cdk4 or Cdk6) and phosphorylate target molecules required for G_1 → S progression. The D cyclins are unique in that they are synthesized in response to growth factor stimulation, and will remain active in the cell as long as the mitotic stimulus is present. Once the cell has passed R in late G_1, the subsequent (sequential) synthesis of appropriate cyclins becomes independent of growth factor stimulation, and is regulated autonomously by the intracellular events of the cell cycle.

REGULATION OF CELL-CYCLE KINASE ACTIVITY

Control of enzyme activity is unique, in that protein levels of the kinase subunit remain constant throughout the cell cycle, and do not require activation from a 'proenzyme' precursor form unlike the activation of the serine proteases of the blood coagulation cascade. Regulation is achieved by varying the availability of the regulatory cofactor, the cyclins, through periodic and cell cycle phase-specific synthesis, and degradation of the appropriate cyclin partners.[8] The periodic accumulation of different cyclins determines the sequential oscillation of kinase activities, which in turn determines the ordering of events of the cell cycle.

Cell-cycle kinase activity is regulated by multiple mechanisms.[9] In addition to the requirement for the appropriate cyclin partner, controlled by cell-cycle specific cyclin synthesis and degradation, the kinase subunit (Cdk) must be phosphorylated and/or dephosphorylated at specific amino acid residues. In addition there are proteins that function as Cdk inhibitors (CDKI) that inhibit

Figure 2. Regulation of cell-cycle kinase activity



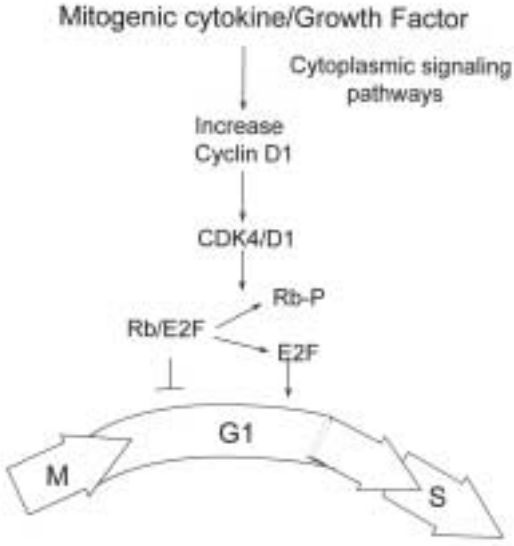
the active kinase activity by binding to the Cdks or the Cdk/cyclin complexes. There are two groups of Cdk inhibitors: the low molecular weight inhibitors (p15, p16, p18, and p19) which primarily inhibit cyclin D/Cdk complexes, and the higher molecular weight inhibitors (p21, p27, and p57) which are universal in their actions and inhibit most of the CDKs (Figure 2).

CELL CYCLE CHECKPOINTS

To assure the orderly progression of cell cycle events, cells use cell cycle checkpoints to monitor events at critical points in the cycle, and if necessary, halt progression of the cycle.[10] The main functions of checkpoints are to detect malfunctions within the system, and to assess whether certain events are properly completed before the cell is allowed to proceed through the cycle. Thus the G_1 checkpoint checks for DNA damage, and will prevent progression into S-phase, if the integrity of the genome is compromised. The G_2-M checkpoint monitors the accuracy of DNA replication during S-phase; it checks for damaged or unreplicated DNA and can block mitosis if any is found. The metaphase checkpoint functions to ensure that all chromosomes are properly aligned on the spindle apparatus prior to initiating chromosomal separation and segregation at anaphase. If defects are detected at any of these checkpoints, the cell cycle is stopped. Activation of DNA repair pathways may be initiated, or if the damage is severe, apoptosis may be triggered.

Although proper function of all three checkpoints is important for assuring the accuracy of the transfer of genetic information, the G_1 checkpoint is particularly important, because once the cell passes R, progression through the rest of the cell cycle becomes independent of external growth regulatory controls. Two proteins critical for effective function of the G_1 checkpoint are p53 and Rb. Rb is the protein product of the retinoblastoma susceptibility gene, which predisposes individuals to retinoblastomas and other tumors when only one functional copy is present. Rb is an important regulator of

Figure 3. Cell cycle checkpoints



cell cycle progression (Figure 3).[11] In its *hypo*phosphorylated (active) state Rb has antiproliferative effects. It inhibits cell cycling by binding transcription factors (the E2F proteins) required for the transcription of genes needed for cell cycling, rendering them transcriptionally inactive. When growth factors induce activation of cyclin D/Cdk4/6, one of the targets of this kinase is the Rb protein. *Hyper*phosphorylation of Rb by cyclin D/Cdk4/6 kinase causes it to dissociate from E2F, which can then activate transcription of genes required for cell cycle progression. Rb thus functions as a tumor suppressor gene, in that cells lacking functional Rb protein show deregulated expression of cell cycle-control genes and cell proliferation, sometimes resulting in malignancy.

Although Rb was the first well-characterized example of a human tumor suppressor gene, p53 has proved more important in human tumorigenesis. The p53 protein operates at the intersection of three important cell processes: control of cell cycle progression, DNA damage recognition and repair, and programmed cell death (apoptosis). P53 is not required for *normal* cell function, i.e., it is not required for cell cycle progression, but serves an important function as a molecular policeman monitoring the integrity of the genome.[12] It is induced in response to DNA damage and puts the brakes on cell growth and division, allowing time for DNA repair or triggering apoptosis if repair is not possible. P53 is an important component of the G_1 checkpoint, although it is also involved in the G_2/M checkpoint as well. Like Rb, p53 functions as a tumor suppressor gene. It is the most commonly mutated gene in human tumors, with mutations of p53 being found in over 50% of all cases of human malignancies.

The acquisition of autonomous or unregulated growth properties is a characteristic of many types of human cancers. Defects in the cell cycle machinery underlie a wide range of malignancies.[13] Cyclins and Cdks may be over-expressed or structurally altered, resulting in increased proliferative activity.[14] Alternatively, the genes encoding CDK inhibitors may suffer a loss of function mutation, which would also contribute to excess or unregulated proliferation. As a result, the cell is no longer able to respond to important internal or external signals that check its growth. Thus many cell cycle regulator genes may be considered proto-oncogenes or tumor suppressor genes. Point mutations, amplifications, deletions, or rearrangements involving their loci are associated with various tumors.

Malignant cells frequently have alterations of genes affecting one or more of the cell cycle checkpoints. Of particular interest are the genes involved in restriction point control (Figure 4). When all components of the Rb pathway are considered collectively, it appears that one or more cell cycle regulators are aberrant in nearly every tumor. It has been proposed by researchers in the field that inactivation of the Rb pathway may well turn out to be an obligatory step in oncogenesis.[15]

Another characteristic of cancer cells is their genetic instability and heterogeneity. Failures of the cell cycle checkpoints, which normally function to ensure genomic integrity, are believed to play a role in the chromosomal and genetic instability of cancer cells. Defects in DNA repair mechanisms also contribute to tumorigenesis in some diseases.

ABNORMALITIES OF DIFFERENTIATION

Malignant tumors often tend to be less well differentiated: they lose some of the specific features of the normal cells from which they originate (anaplasia).

In normal tissues, differentiation may be accompanied by an irreversible commitment to a nonproliferative, post-mitotic state.

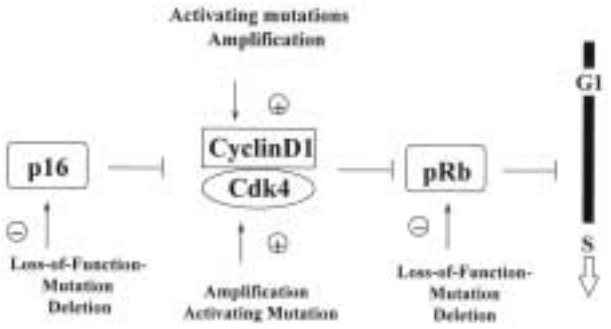


Figure 4. Perturbation of the p16-cyclinD1-Rb pathway in cancer cells. Restriction point control

Many cells, such as hematopoietic cells, must withdraw from the cell cycle, i.e., enter the G_0 state so that they can differentiate. In a way, differentiation and cell division can be viewed as alternative transcriptional programs. If the cell differentiates, certain genes are turned on, whereas the genes involved in cell cycle progression are repressed. Conversely, if the cell proceeds through the cell cycle, the genes required for proliferation are induced, whereas genes required for differentiation are repressed. In many tissues, the activation of growth and the induction of the cell cycle program can lead to the suppression of differentiation. The abnormalities in cell differentiation that are seen in many tumor cells may result, at least partly, from the mutations responsible for the activation of autonomous cell growth.

APOPTOSIS (PROGRAMMED CELL DEATH)

Tissue homeostasis depends not only on the rate of cell proliferation, but also on the rate of cell death. The growth of tumors may depend not only on an increase in the fraction of cells that are proliferating, but also on a decrease in the fraction of cells that are undergoing programmed cell death.

Cells can die by two major mechanisms: either necrosis or apoptosis. The criteria for determining whether a cell is undergoing apoptosis or necrosis originally relied on distinct morphological changes in the appearance of the cell.[16] However, apoptotic changes can now be defined at the molecular level, and apoptotic cells can be detected by a variety of biochemical and immunological laboratory methodologies.

Necrotic cell death is induced by lethal chemical, biological or physical events (extracellular assault). Such a death has been described as analogous to 'cell murder'. In contrast, apoptosis or 'programmed cell death' requires the coordination of gene-directed internal processes, and is analogous to 'cell suicide', as death is the consequence of molecular signals contained within individual cells.

Apoptosis is now recognized to be essential in the development and homeostasis of all multicellular organisms.[17] Apoptosis plays a dominant role in the development of the organism (embryogenesis/organogenesis), in tissue homeostasis (to remove excess or unwanted cells following an expansion stimulus, e.g., expanded T or B lymphocytes following immune stimulation), and as a defense mechanism, to remove unwanted and potentially dangerous cells such as self-reactive lymphocytes, virally-infected cells, or tumor cells. Cells that have sustained genotoxic injury, if they are unable to repair the damage to their DNA, will initiate a 'self-suicide' program to prevent the cell with damaged DNA from proliferating. The failure of certain tumor cells to undergo apoptosis contributes to the genetic instability of some cancer cells.

CASPASES AND THE REGULATION OF APOPTOSIS

The cellular events responsible for apoptotic cell death are directed by a group of proteins called caspases.[18] Caspases are a family of cysteine proteases that cleave after aspartic acid amino acids in a

peptide substrate, and are responsible for the orderly dismantling of the cell undergoing apoptosis. Caspases form the effector arm of the apoptotic machinery that, once activated, carry out the proteolysis necessary for apoptosis to occur. There is a hierarchical relationship described among the various apoptotic caspases, somewhat analogous to that described for the blood coagulation proteases. Early acting, 'initiator' caspases (caspase-2, -8, -9, -10) are recruited in response to apoptotic stimuli and are activated. They then initiate the cascade by activating downstream 'executioner' caspases (caspase-3, -6, -7), which activate pro-apoptotic factors, cleave key proteins required for maintenance of intracellular homeostasis, and orchestrate the ordered dismantling of the cell (Figure 5). Activation of caspases in apoptosis does not lead to indiscriminate proteolytic degradation, but rather specific cleavage of key substrates including proteins involved in cell structure, proteins involved in cell cycle regulation, DNA repair proteins, proteins involved in RNA splicing, and the activation of a key endonuclease responsible for the characteristic DNA fragmentation.

Apoptosis is a closely regulated physiologic process. A number of proteins that modulate cell death by interfering with caspase activity have been described, the inhibitors of apoptosis proteins (IAPs).[18] The Bcl-2 family of proteins is particularly important in regulating apoptosis.[19] This 'family' of proteins includes both pro-apoptotic (Bax, Bad, et al.) and anti-apoptotic (Bcl-2, Bcl-X_L, et al.) members, and constitutes a critical intracellular checkpoint of apoptosis, determining whether early activation of initiator caspases will proceed to full activation of execution caspases and cell death.

Bcl-2 is a protein originally cloned from B-cell lymphomas with the characteristic t(14;18) chromosomal translocation. Since that initial discovery, several additional related proteins have been identified, including some with proapoptotic activity and others that are antiapoptotic. These proteins are thought to function through protein-protein interactions. They all share similar structural regions that allow them to form dimers, either homo- or heterodimers. Bax, the first pro-apoptotic member discovered, can heterodimerize with Bcl-2 (an anti-apoptotic protein) or homodimerize with itself (Figure 6). Bax:Bax homodimers promote apoptosis; with elevated levels of Bcl-2, Bax:Bcl-2 heterodimers form, which repress apoptosis. Actually, it is the overall ratio of death agonists (Bax and related proteins) to death antagonists (Bcl-2 and related proteins) that determines the susceptibility to a death stimulus.

Dysregulation of apoptosis represents a significant feature of many malignancies.[20] The acquisition of genetic changes that are anti-apoptotic is crucial to the development of many leukemias and other malignancies. The t(14;18) translocation in follicular lymphoma results in constitutive expression of Bcl-2, which functions to protect the malignant cells against cell death in response to many stimuli. Subsequent to its identification in B-cell follicular lymphoma, elevated Bcl-2 has been shown to play a role in a wide range of human cancers.[21,22] Overexpression of Bcl-X_L (also antiapoptotic) has been demonstrated in the erythroid precursors

Figure 5. Death receptor/death cytokine apoptotic pathway. Caspases and the regulation of apoptosis

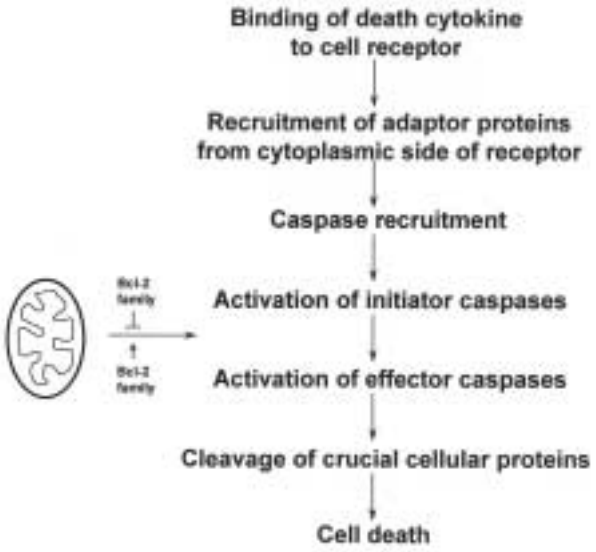
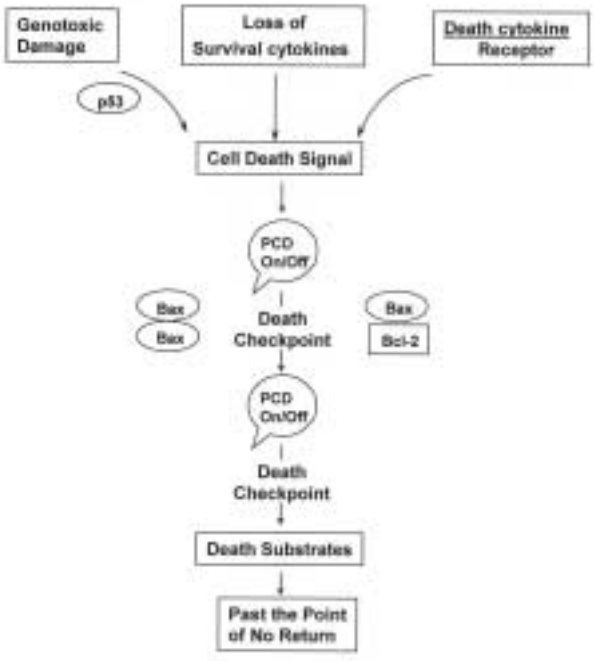


Figure 6. Role of Bax in apoptosis



of patients with polycythemia vera. Just as over-expression of antiapoptotic proteins is associated with many human cancers, loss-of-function abnormalities in pro-apoptotic proteins are an important alternative mechanism of oncogenesis.

Alternatively, excess apoptosis has been suggested to contribute to the pathogenesis of a number of diseases characterized by cytopenias, including aplastic anemia, Diamond-Blackfan anemia, and the myelodysplastic syndromes.[23]

TELOMERES AND TELOMERASE

Normal human cells have a finite proliferative life-span, and enter a nondividing state termed senescence. Replicative senescence is dependent upon cumulative cell divisions. Because of a quirk, the enzyme that duplicates DNA before cells divide cannot replicate the entire length of both strands of DNA. The conventional DNA replication mechanism does not synthesize the very end of the chromosome. As a result, chromosomes shorten with each successive round of cell division.

Telomere loss is thought to control entry into senescence. Telomeres consist of repetitive DNA sequences that cap the ends of chromosomes, and which shorten each time the cell divides. These repeats are synthesized by the ribonucleoprotein telomerase. After birth, somatic cells of humans shut off telomerase activity, and consequently, successive rounds of cell division result in telomere shortening. When several kilobases of the telomeric DNA have been lost, the telomeres reach a critically short length and the cells stop dividing and go into senescence.

In contrast to most somatic cells, the vast majority of human tumors expresses telomerase, and hence are able to maintain telomeric DNA (a crucial step in tumorigenesis?).[24] It has been shown that telomerase reactivation occurs in many different cancers, and functions to rebuild the telomeres after each cell division, keeping the cell immortal. In one study, telomerase was shown to be active in nearly 85% of primary tumors; including cancers of the breast, brain, and lung.[25] This finding has prompted some investigators to propose that sustained growth of a cancer requires the reactivation of telomerase.

CONCLUSION

Thus, diverse mechanisms are capable of inducing genetic alterations that may uncouple the cell from its normal growth controls, resulting in excessive or improperly regulated cell growth or apoptosis. Many studies support the concept that cancer, in most instances, represents a multistep process, resulting from a series of genetic changes. Ongoing research is continuing to delineate the interaction of these various pathways, and attempting to clarify the molecular mechanisms underlying phenotypic evolution of malignancies. The increased understanding of the 'molecular logic' behind these various cellular processes is resulting in improved methods for diagnosis and prognosis of disease. The ultimate goal is that by elucidating the fundamental processes that lead to neoplastic disease, we will eventually have the knowledge to control it. Significantly, researchers are designing novel drugs and gene therapy strategies to treat these disorders.

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