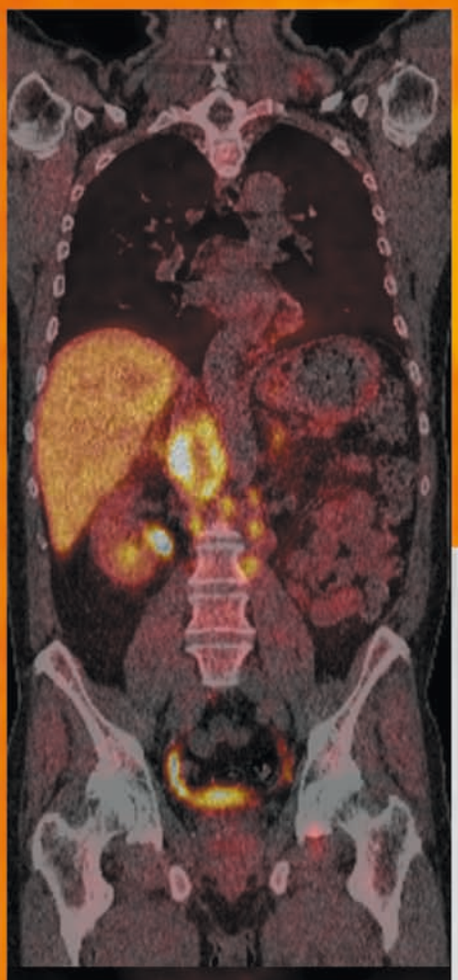

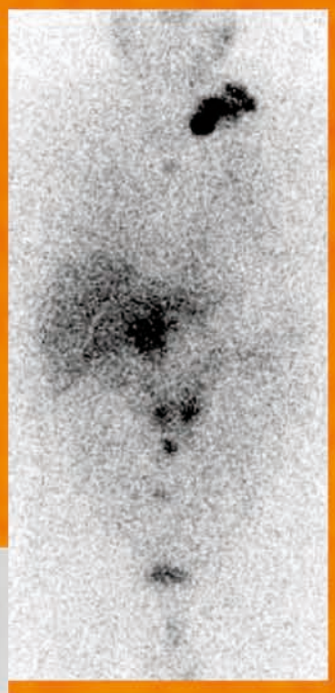

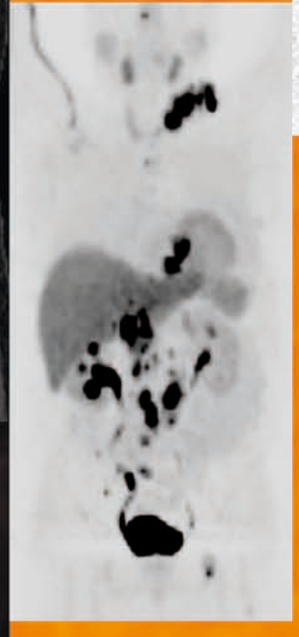

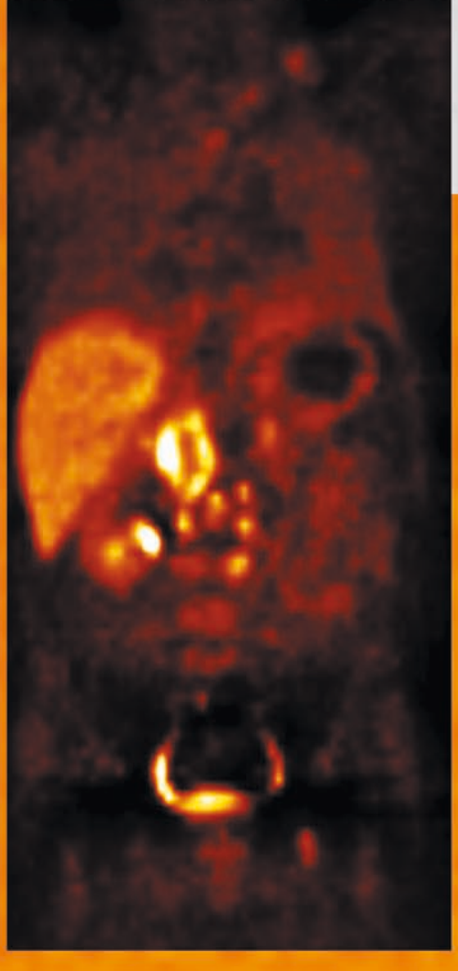

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Diagnosis and Therapy

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Preface

There has been enormous progress in nuclear medicine in recent years, and the impact of this progress has been particularly noticeable in oncology. Research into molecular imaging has led to the development of radiopharmaceuticals that can explore the cellular metabolism, and visualize at molecular and subcellular levels the pathological processes specific to cancer. Equipment development has produced high-technology instruments such as those used in positron emission tomography (PET), able to produce high-quality images that have become indispensable in the diagnostic work-up of cancer patients, because they often reveal alterations and lesions not demonstrated by conventional morphologically oriented techniques such as X-ray imaging, ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI). Research in the area of image fusion techniques has led to the design of software programs able to merge in a single image the molecular, functional, and metabolic information of nuclear medicine with the morphological information provided by radiology. Hybrid instruments (PET/CT, SPECT/CT) are now available which allow the fusion of images of a patient in just one diagnostic session. Intensive research is ongoing to obtain detectors, hardware, and software able to perform whole-body scans faster and with increasing spatial resolution, so that it may become possible to detect lesions on a submillimeter level. Nuclear medicine has made the step from bench to bedside, to a significant extent.

All of these achievements have had a great impact on not only the diagnosis but also the treatment of cancer. Improved, individually tailored therapy is now on the horizon. Radiopharmaceuticals developed specifically to target and visualize malignant tumors can also be used, at high doses, for therapeutic purposes. Nuclear medicine therapeutics thus takes advantage of selective radiopharmaceuticals that have demonstrated marked anticancer efficacy in many types of tumors. For example, in recent years, these techniques have been used and shown greatest efficacy in the treatment of lymphomas and neuroendocrine tumors.

The diagnostic and therapeutic achievements in nuclear medicine are the result of the interdisciplinary research efforts of cell biologists, chemists, pharmacologists, physicists, computer scientists, engineers, nuclear medicine physicians, and oncologists. The clinical implications of these achievements have made nuclear medicine indispensable in the management of cancer.

This textbook on modern nuclear medicine applications in the diagnosis and treatment of cancer describes the state of the art and the current position of nuclear medicine in the light of these recent developments. It is intended as a valuable update also for non-nuclear medicine specialists working in oncology. Nuclear medicine as part of molecular imaging and therapy has changed radically in the past decade. The growing importance and clinical impact of these changes for the near future has impelled the authors to record them in this book.

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1

What is cancer?

Uwe Haberkorn

At first sight cancer is a disease induced by the failure of control mechanisms. The cancer cell does not respond to control signals because of damage to its DNA, the presence of oncogene products, or because the homeostatic control mechanisms themselves are disturbed. Biologically this corresponds to uncontrolled proliferation occurring at the wrong place and time driven by oncogenic signals, impaired differentiation, and invasion of other tissues leading to metastases.

In a recent review, Hanahan and Weinberg mentioned six essential alterations in cell physiology which are seen as the hallmarks of cancer: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. These alterations are interpreted as results of genetic changes in the cancer cell. However, mutations in the tumor genome may not be the only cause.[1]

Genetic and epigenetic background

Cancer has been viewed as a multistep process of genetic alterations that result in the transformation of benign cells into malignant ones. These genetic abnormalities include mutations in tumor-suppressor genes and oncogenes, and chromosomal abnormalities such as chromosomal gain, loss, and/or rearrangement (Table 1.1).[1,2] Such events are thought to be followed by a clonal selection of variant cells that show increasingly aggressive behavior.[3]

Although it is still commonly thought that aneuploidy occurs as a late-stage effect rather than as a cause of cancer development, this might not always be true, as carcinogens such as asbestos and arsenic initially do not cause gene mutations, but rather lead to aneuploid lesions. Furthermore, normal cells exposed to chemical carcinogens can become aneuploid long before they show signs of being cancerous. Therefore, it is possible that gains and losses of whole chromosomes may disturb the balances that regulate normal growth control. On the other hand, many normal cells, both in vitro and in vivo, may become cancerous after the right combination of oncogenes is introduced.[4] However, only a fraction of these cells will give rise to cancer, implying that other yet unknown factors might also be involved in tumor initiation. Therefore, both mutations and chromosomal derangements are important in the initial stages of tumor development, and both mechanisms might be involved in establishment of the cancer stem cell.

Many of the oncogenes act by mimicking normal growth signaling. This can be accomplished by alteration of extracellular growth signals, or alterations of transcellular transducers of those signals or of intracellular circuits that translate those signals into cellular response. Many cancer cells acquire the ability to synthesize growth factors to which they are responsive, creating a positive feedback signaling loop. Examples of this autocrine stimulation are platelet derived growth factor (PDGF) and transforming tumor growth factor β (TGFβ). Furthermore, there is overexpression of growth factor receptors, which often carry tyrosine kinase activities in their cytoplasmic domains. This results in cells becoming hyperresponsive even to low growth factor levels that normally would not trigger proliferation. As an example, members of the epidermal growth factor receptor family such as EGFR/erbB are upregulated in non-small-cell lung cancer (NSCLC) and head and neck, renal cell, brain, and breast tumors, and HER2/neu receptors are overexpressed in stomach and mammary tumors. Ligand independent signaling can also be achieved through structural alteration of receptors: truncated versions of the EGFR lacking parts of the cytoplasmic domain act constitutively. Finally, there are alterations of the downstream cytoplasmic signaling pathways, which receive and process the signals emitted by ligand-activated growth factor receptors and integrins. In that respect, the Ras–Raf–MAPK (mitogen-activated protein kinase) cascade plays a central role. In about 25% of human tumors, Ras proteins are present in structurally altered forms. This has been exemplified in human colon carcinomas where about 50% of the tumors bear mutant

Table 1.1 Regulatory proteins for tumorigenesis, apoptosis, and drug resistance

Protein	*Role in tumorigenesis, apoptosis, and drug resistance*
Suppressor proteins	
p53	Mutated or altered expression in many cancers. Initiates the intrinsic apoptotic pathway. p53-negative cells are resistant to drug-induced apoptosis
ATM	Mutated in ataxia–telangiectasia syndrome. Senses DNA double strand breaks and stabilizes p53. Increased risk for hematological malignancies and breast cancer
CHK2	Mutated in Li–Fraumeni syndrome. Senses DNA double strand breaks and phosphorylates and stabilizes p53
Rb	Mutated in some cancers, functionally disrupted in many cancers. Inhibits E2F-mediated transcription. Loss of Rb function induces p53-dependent and -independent apoptosis
Bax	Mutated or decreased expression in some tumors. Mediates mitochondrial membrane damage
Bak	Mutated or decreased expression in some tumors. Mediates mitochondrial membrane damage
PTEN	Mutated or altered expression in cancers. Regulates Akt activation and subsequent phosphorylation of Bad. Loss of PTEN results in resistance to many apoptotic stimuli
APAF1	Mutated and transcriptionally silenced in melanoma and leukemia cell lines. Necessary for activation of caspase-9 following cytochrome c release. Chemoresistance
CD95/Fas	Mutated and downregulated in lymphoid and solid tumors. Initiates the extrinsic apoptotic pathway. Resistance to drug-induced cell death
TRAIL-R	Mutated in metastatic breast cancers. Initiates the extrinsic apoptotic pathway. Mutations lead to suppression of death receptor-mediated apoptosis
Caspase-8	Silenced in neuroblastomas. Activates both extrinsic and intrinsic apoptotic pathways. Resistance to drug-induced apoptosis
Oncogenes	
Bcl-2	Frequently overexpressed in many tumors. Antagonizes Bax and/or Bak and inhibits mitochondrial membrane disruption. Inhibits drug-induced apoptosis
MDM2	Overexpressed in some tumors. Negative regulator of p53. Inhibits drug-induced p53 activation
IAPs	Frequently overexpressed in cancer. Downregulation of XIAP induces apoptosis in chemoresistant tumors
NFκB	Deregulated activity in many cancers. Transcriptionally activates expression of antiapoptotic members of the Bcl-2 and IAP families. Can inhibit both the extrinsic and intrinsic death pathways and induce drug resistance
Myc	Deregulated expression in many cancers. Induces proliferation in the presence of survival factors, such as Bcl-2, and apoptosis in the absence of survival factors. Can sensitize cells to drug-induced apoptosis
Akt	Frequently amplified in solid tumors. Phosphorylates Bad. Hyperactivation induces resistance to a range of apoptotic stimuli
PI3K	Overexpressed or deregulated in some cancers. Responsible for activation of Akt and downstream phosphorylation of Bad. Inhibition of PI3K enhances chemotherapeutic drug-induced apoptosis
Ras	Mutated or deregulated in many cancers. Activates PI3K and downstream pathways. Induces proliferation and inhibits c-myc and drug-induced apoptosis

ATM, ataxia telangiectasia mutated; Chk2, checkpoint kinase 2; PTEN, phosphatase and tensin homolog; Apaf-1, apopotic protease activating factor 1; TRAIL-R, TNF-related apoptosis-inducing ligand receptor; MDM2, transformed 3T3 cell double minute 2; IAPs, inhibitor of apoptosis proteins; NFκB, nuclear factor κB; Akt, protein kinase B; PI3K, phosphatidylinositol 3-kinase.

ras oncogenes. It is suggested that the remaining colonic tumors carry defects in other components of the growth signaling pathways, with similar functional results to those obtained after ras oncogene activation.[1]

Besides response to growth signals, resistance to antigrowth signals is an equally important feature of cancer. These antiproliferative signals are coordinated mainly by the retinoblastoma protein (pRb) and its two relatives p107 and p130. Hypophosphorylated pRB blocks proliferation by sequestering and altering the function of E2F transcription factors that control the expression of several genes which are essential for the progression from G1 into S phase.[5] Disruption of the pRb pathway liberates E2F and allows cell proliferation, rendering cells insensitive to antigrowth factors. In this respect, transforming growth factor β (TGFβ) is an important regulator of pRb modification by preventing the inactivating phosphorylation of the protein. Response to TGFβ can be lost after downregulation of TGFβ receptors, or mutant, dysfunctional receptors.[6] In addition, changes in the signaling pathway may occur: the function of proteins such as Smad4, which transduces signals from ligand-activated TGFβ receptors to downstream targets, or p15INK4B may be changed by mutation of the corresponding genes.[7,8]

Differentiation is is also a condition which results in the inhibition of proliferation and is disturbed in a variety of tumor cells. One of the target genes in this respect is the c-myc oncogene, which encodes a transcription factor. During normal development, the growth-stimulating action of Myc, in association with another factor, Max, can be inhibited by the formation of complexes of Max with a group of Mad transcription factors. These Mad–Max complexes result in differentiation-inducing signals.[9] Overexpression of the c-Myc oncoprotein occurs in many tumors and shifts the balance to Myc–Max complexes, which impairs differentiation and thereby promotes tumor growth. A further example is inactivation of the APC/β-catenin pathway in colon carcinoma, which results in a block of the differentiation of enterocytes in the colonic crypts.[2]

The characteristics mentioned above are subsumed under the term 'somatic mutation theory of carcinogenesis', which has been the dominant force driving cancer research during the 20th century. In brief, it proposes that successive DNA mutations in a single cell cause cancer. This theory places carcinogenesis at the cellular and subcellular hierarchical levels of biological complexity. However, increasing evidence has been obtained that epigenetic changes and also changes in surrounding or tumor infiltrating non-tumor cells such as fibroblasts and endothelial cells are important. These may interact with tumor cells by secretion of a variety of signaling factors such as diffusible growth factors, extracellular matrix components, or cell-to-cell adhesion/interaction molecules. Evidence of a promotion of cancer cells by inflammatory cells infiltrating the tumor site has also been found.[10]

Epigenetic changes are realized by three different mechanisms: DNA methylation, RNA-associated silencing, and histone modification, which are known to initiate and sustain epigenetic silencing, and to interact with each other.[11–13]

Methylation of the C5 position of cytosine residues in DNA is maintained by a number of DNA methyltransferases and has multiple roles for the silencing of transposable elements, for defense against viral sequences, and for the transcriptional repression of genes. The resulting metabolite, 5-methylcytosine, is highly mutagenic, causing C:G to T:A transitions, and leads to a suppression of the methylated site in the human genome. The predominant sites, CpG islands, are regions of more than 500 base pairs in size and with a GC content greater than 55%,[14] and have been conserved during evolution because they are normally kept free of methylation. They are located within the promoter regions of about 40% of mammalian genes and can be transcriptionally silenced by methylation. Extensive de novo methylation of CpG islands is a common feature of many cancers.[15]

Histone modifications such as acetylation, phosphorylation, and methylation of conserved lysine residues on the amino-terminal tail domains have also been defined as epigenetic modifiers. Acetylation of histones causes transcriptionally active DNA regions, whereas hypoacetylated histones are associated with transcriptionally inactive DNA regions. Since there is a considerable variation of all possible histone modifications, and also interactions between histone deacetylases, histone methyltransferases, and methylcytosine-binding proteins occur, this is seen as a histone code which is used by a variety of cellular factors.[16,17]

The role of epigenetic changes in cancer has been shown for the MLH1 gene, where methylation and silencing of the gene may lead to a variety of cancers.[18,19] Chromatin-modifying enzymes have also been associated with human leukemias, with histone acetyltransferases and histone methyltransferases engaged in the modification of fusion protein activity, such as the oncogenic PML–RARα (promyelocytic leukemia–retinoic acid receptor α) in acute promyelocytic leukemia, which recruits a histone deacetylase to repress genes essential for the differentiation of hematopoietic cells, or AML1-ETO (AML refers to acute myeloid leukaemia) fusions, which recruit a histone deacetylase 1 complex to inhibit myeloid development.[20,21] Furthermore, loss or mutations of adenosine triphosphate (ATP)-dependent chromatin remodeling complexes such as SWI–SNF, BRM, and BRG1 have been found to be associated with pediatric cancer as well as a variety of cancer cell lines and tumor tissues.[22]

A simple way to induce a carcinogenic phenotype is the transcriptional repression of tumor suppressor genes, which may represent an alternative mechanism to genetic mutation. In addition, cancer-cell genomes simultaneously show global hypomethylation and gene promoter-specific

hypermethylation. This might contribute to genomic instability, structural changes in chromosomes, and increases in gene expression.[15,23–25] These alterations may occur at early stages of carcinogenesis and may determine the subsequent genetic changes and progression of these mutated clones.

Many genes are epigenetically silenced in cancer cells, and many epigenetically silenced genes have not been found to contain any genetic mutations at all, even though they are transcriptionally repressed in many different cancer-cell types. These facts underscore the potential value of screening for all epigenetic modifications, as well as genetic changes, that are associated with human tumor types. Examples of a combination for genetic and epigenetic changes were found in the colon cancer cell line HCT116, which contains several mutations, including the DNA mismatch-repair protein, MLH1, and p16, which contribute to the mismatch-repair phenotype and to disruption of the cyclin D-RB1 (retinoblastoma 1) cell-cycle-control pathway, the transforming growth factor-β2 receptor, which causes a loss of control of a cell differentiation pathway, and an activating mutation in the gene that encodes β-catenin, resulting in constitutive Wnt (wingless type) signaling and cell proliferation.[26–30] In addition to these mutations, there are at least 14 epigenetically silenced genes in these cells, all of which can be reactivated by either treating the cells with DNA-demethylating agents or disrupting the genes that encode DNA methyltransferases, which catalyze DNA methylation.[31–35] Reactivating expression of these growth-control genes results in phenotypic changes that range from reducing proliferation to inducing senescence or apoptosis.[26,27,33] Epigenetic alterations of these genes seem to complement mutations in determining the phenotype of these cells. Examples of a collaboration between epigenetic and genetic abnormalities are MLH1 and CDKN2A (the gene that encodes p16) in HCT116 cells: while one allele of each of these genes is mutated in these cells, the wild-type allele becomes silenced by hypermethylation. Therefore, genetic and epigenetic changes can collaborate to prevent expression of a functional gene product in cancer cells.

Another epigenetic–genetic collaboration in HCT116 cells is found in the Wnt pathway. Four members of the secreted frizzled-related gene family (SFRP1, SFRP2, SFRP4, and SFRP5) that encode Wnt antagonists are epigenetically silenced in these cells. This contributes to the abnormal activation of Wnt signaling, even in cells that already carry activating mutations in β-catenin.[33] In addition, silencing of the genes that encode the transcription factors GATA4 and GATA5, as well as their downstream activation targets trefoil factor 1 (TFF1), TFF2, TFF3, and inhibin-α,[32] could impair maturation of endoderm-derived epithelial cells.[36–39] Finally, TIMP3 (tissue inhibitor of metalloproteinase 3) is silenced in HCT116 cells, and loss of function of its product might increase the invasive ability of these cells.[35]

Epigenetic silencing may occur during the early stages of tumor progression, possibly during the abnormal expansion of stem and progenitor cells. This silencing predisposes the stem cells to abnormal clonal exposition. Changes that are known to contribute to tumor formation such as chronic inflammation, which leads to the production of reactive oxygen species, induce cell renewal dedicated to repair of tissue damage. This is associated with epigenetic events which lead to heritable transcriptional repression and activation of stem-cell and progenitor-cell expansion at the expense of normal cell differentiation and maturation. The subsequent progression to malignancy would then depend not only on gene mutations but also on the collection of epigenetic alterations. These epigenetic changes might occur continuously not only in epithelial cells but also in surrounding stromal cells.[40]

The reductionist approach, which sees cancer solely as a disease reducible to mutations, has been challenged not only by the finding of epigenetic changes but also by the number of mutations occurring in cancers. These range from three to anywhere from 11 000 to 100 000 mutations.[41–43] In principle these data, obtained from high-throughput technologies such as gene arrays, may be used to introduce new classifications.[44] However, these new technologies face problems associated with bias, reproducibility, overfitting, and data interpretation.[45]

This has led to the proposal of an alternative theory, the tissue organization field theory of carcinogenesis and neoplasia. Its assumptions are that proliferation is the default state of all cells and that carcinogenesis and neoplasia are defects of tissue architecture. Carcinogens would act initially by disrupting the normal interactions between parenchymal cells and the stroma of the organ. In this model the stroma appears as the primary target of carcinogens, stating that carcinogenesis and neoplasia occur exclusively through supracellular phenomena. This implies that neoplastic cells may be reprogrammed to behave as non-tumor cells when placed in the context of normal tissues.[46] Evidence is derived from early studies with teratocarcinomas, where the stem cells generated not only more stem cells but also more differentiated cells that gave rise to non-tumorigenic tissue. The teratocarcinoma cells were generated from tumors resulting from the implantation of normal embryos into ectopic locations. Teratocarcinoma cells injected into normal blastocysts were shown to generate normal tissues in viable mosaic individuals resulting from this manipulation.[47,48] In subsequent generations, normal offspring resulted from the genome of a cell that was once a cancer cell. If cancer indeed results from the accumulation of DNA mutations in a previously normal cell, it becomes problematic to explain these data. More recently, ectopic expression of stromelysin-1 by mammary gland epithelial cells in transgenic animals resulted in mammary gland carcinoma. In this case, expression of this enzyme induces stromal changes that in turn would lead to carcinoma.

Treatment with specific protease inhibitors blocked carcinogenesis in this model.[49] Also, irradiation of the stroma of epithelium-free mammary glands results in carcinogenesis in non-irradiated mammary epithelial cells inoculated into the irradiated stroma.[50] In all these experiments, the parenchyma–stroma interaction seems to be an important factor for cancer progression.[51,52] From the evidence mentioned above it follows that cancer is more than a disease of specific genes. Rather, instability of the genome as a whole must be seen as a hallmark of malignant tumors.[53–56] Furthermore, the concept that epigenetic abnormalities could be as important as genetic ones in determining the course of tumor development, and also be involved in tumor-specific signaling pathway abnormalities, is relevant to the future design of both preventive and therapeutic approaches to cancer.[13]

Cell death: Apoptosis and more

To control abnormal proliferation a cell can either enter a quiescence-like growth arrest phase, or undergo apoptosis or senescence. These antiproliferative programs are induced by tumor suppressors such as p53 and pRb in response to potential oncogenic signals.

The most common and well-defined form of programmed cell death is apoptosis, which is a physiological cell-suicide program that is essential for embryonic development, function of the immune system, and the maintenance of tissue homeostasis in multicellular organisms. Apoptosis in mammalian cells is mediated by a family of cysteine proteases known as the caspases. To keep the apoptotic program under control, caspases are initially expressed in cells as inactive procaspase precursors. When initiator caspases such as caspase-8 and caspase-9 are activated by oligomerization, they cleave the precursor forms of effector caspases, such as caspase-3, caspase-6, and caspase-7.[57,58] Activated effector caspases cleave a specific set of cellular substrates, resulting in specific biochemical and morphological changes (Table 1.2) that are associated with the apoptotic phenotype. Dysregulation of apoptosis has been implicated in numerous pathological conditions, including neurodegenerative diseases, autoimmunity, and cancer.[59]

There are two pathways by which caspase activation is triggered, the extrinsic and intrinsic apoptotic pathways. The extrinsic pathway is induced by activation of death receptors on the cell surface. Binding of ligands such as FasL and tumor necrosis factor (TNF) to Fas and the TNF receptor (TNFR), respectively, leads to formation of the death induced signaling complex (DISC). DISC recruits caspase-8 and promotes the cascade of procaspase activation (Figure 1.1).[60] The intrinsic pathway is triggered by various extracellular and intracellular stresses, such as growth-factor withdrawal, hypoxia, DNA damage, and

Table 1.2 Morphological and biochemical differences between apoptosis and necrosis

Features	*Apoptosis*	*Necrosis*
Tissue distribution	Single cells	Multiple cells
Tissue reaction	Phagocytosis	Cellular exudate
Cell morphology	Shrinkage	Swelling
Organelles	Intact	Damaged
Chromatin	Marginated, condensed	Fragmented
Membrane	Intact	Damaged
Biochemistry	Activated endonucleases DNA cleavage Activated caspases	Defective ion pump Activated lysosomal enzymes

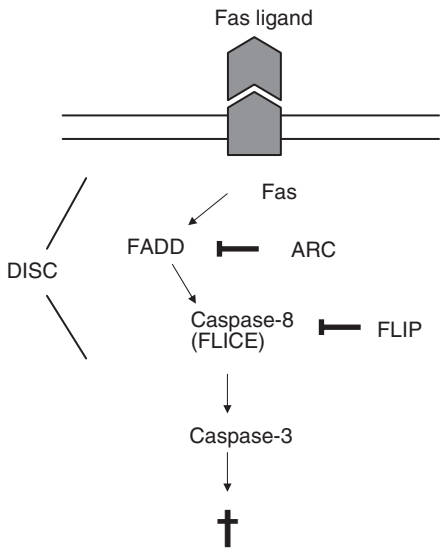

Figure 1.1
Simplified scheme of the death receptor-mediated or extrinsic apoptosis pathway. Binding of the Fas ligand is followed by formation of the death inducing signaling complex (DISC): the adaptor molecule FADD binds to the death domain of Fas and recruits procaspase-8 via its death effector domain. Autocatalytic activation of procaspase-8 leads to activation of the downstream effector caspase-3. The cascade may be inhibited by FLICE inhibitory protein (FLIP) or by the apoptosis repressor with a CARD domain (ARC).

oncogene induction. Signals that are transduced in response to these stresses converge mainly on the mitochondria. A series of biochemical events is induced that results in the permeabilization of the outer mitochondrial membrane, the release of cytochrome c and other proapoptotic molecules, the formation of the apoptosome, a large protein complex that contains cytochrome c, apoptotic protease activating factor 1 (APAF1), and caspase-9, and caspase activation. Once cytochrome c is released, the downstream cascade of caspase activation is irreversible. Cell death is also modified by other proteins such as endonuclease G21 and apoptosis-inducing factor (AIF), which may induce cell death independently of caspase activation.

In order to survive, tumor cells need to avoid apoptosis that can be induced by unregulated oncogene expression, and a limited supply of growth factors, oxygen, or nutrients. Protection from apoptosis may be achieved by modification of the activities of antiapoptotic genes such as Bcl-2 or suppressor genes such as p53. In human B-cell follicular lymphomas, a chromosomal translocation linking the Bcl-2 gene to an immunoglobulin locus was identified in the transformed cells, resulting in constitutively active Bcl-2 and survival of the lymphoma cells.[61]

The tumor suppressor p53 is an important regulator of apoptosis and is the most commonly mutated gene in cancer.[62] Normal function of p53 induces apoptosis in the presence of genotoxic stress, causing DNA damage that cannot be repaired during cell-cycle arrest, growth-factor withdrawal, hypoxia, and dysregulated expression of mitogenic oncogenes. In addition to the fact that most human cancers have either mutations in p53 or defects in the pathway, p53-null mice are highly prone to developing cancers.

As the Fas pathway regulates the immune system through its proapoptotic function, disruption of this pathway may lead to lymphoproliferative disorders and hematopoietic cancers. Somatic mutations of the Fas gene or its downstream effectors have been found in patients with multiple myeloma, non-Hodgkin's lymphoma, and other cancers.[63,64] Alterations to cell-survival pathways may also be involved in the suppression of apoptosis. The PI3K–Akt (phosphatidylinositol 3-kinase–protein kinase B) survival signaling pathway is activated by various intracellular and extracellular stimuli and modulates apoptotic pathways, resulting in the resistance of tumor cells to death signals. Akt signaling induces expression of the antiapoptotic molecule Bcl-XL, inhibits the proapoptotic activity of FKHRL1 (FOXO3A), and leads to negative regulation of p53 mediated apoptosis.[65] Akt activation also provides cells with a survival advantage through its promotion of glucose metabolism.[66,67] Another survival factor that is relevant to human tumorigenesis is nuclear factor κB (NFκB), a transcription factor that is activated by numerous cytokines and oncogenes. De novo gene transcription that is induced by NFκB prevents apoptosis that is induced by the engagement of death receptors.[68] Therefore, many different modes of inactivating proapoptotic signaling pathways underlie tumorigenesis.

Several non-apoptotic cell death mechanisms have been identified, including necrosis, autophagy, mitotic catastrophe, and senescence. In contrast to apoptotic cell death, necrosis is an unregulated process with membrane distortion, organelle degradation, and cellular swelling, resulting in cell destruction and the release of intracellular components. Necrosis is usually a consequence of a pathophysiological condition, such as infection, inflammation, or ischemia, which leads to the failure of normal physiological pathways that are essential for maintaining cellular homeostasis, such as regulation of ion transport, energy production, and pH balance.

Primary cells in culture initially proliferate rapidly, with a significant shortening of the telomeres of their chromosomes. This may lead to a form of permanent cell-cycle arrest that has been described as replicative senescence. A senescent cell is characterized by flattened cytoplasm, increased granularity, changes in metabolism, and the induction of senescence-associated β-galactosidase activity. Furthermore, alterations in chromatin structure and gene-expression patterns are seen. The phenomenon is inducible by various cellular stresses, DNA damage, and oncogene activity.[69] The senescence program then induces the activation of various cell-cycle inhibitors and requires the functions of p53, the CDKN1A gene product WAF1/p21, the CDKN2A gene product INK4A/p16, and the retinoblastoma protein (pRb). The involvement of these tumor suppressors implies that one of the main functions of the senescence program is to suppress tumorigenesis, a hypothesis that has been confirmed in mutant mice.[70,71]

In normal cells, unwanted proteins or proteins that are no longer required are degraded by two independent mechanisms: ubiquitin mediated proteolysis in proteasomes, and autophagy, a mechanism by which long-lived proteins and organelle components are directed to and degraded within lysosomes.[72] Autophagy is conserved in various species, and is activated in response to growth-factor withdrawal, differentiation, starvation, and stress. After the induction of autophagy, autophagic vesicles (autophagosomes) are formed by the assembly and expansion of membrane-bound structures, probably originating in the endoplasmic reticulum around organelles and isolated proteins. The autophagosome encapsulates the cytosolic materials and fuses with lysosomes or other vacuoles, causing degradation of its content. The signaling pathway that leads to autophagy involves at least the activities of phosphatidylinositol 3-kinase (PI3K) and the kinase target of rapamycin (TOR).[73] The TOR pathway coordinates signaling pathways that are initiated by nutritional and mitogenic factors, and also controls both protein synthesis and degradation. Although the components of the autophagic machinery are highly conserved in a wide range of organisms, the physiological role of the process varies. There is evidence that lysosomal

degradation of organelles is required for cellular remodeling due to differentiation, stress, or damage following exposure to cytotoxins, and that dysregulation of autophagy can result in pathological states such as neurodegenerative diseases, cardiomyopathy, and cancer.[74]

Uncontrolled protein degradation by the proteasomal pathway can contribute to tumorigenesis. Examples are the Wnt and hedgehog (HH) signaling pathways, which are regulated by the turnover of β-catenin and cubitus interruptus (CI). Mutations in the corresponding genes that lead to constitutive activation of Wnt and HH pathways are common in human colon cancer and basal-cell skin carcinomas.[75] Defects in the autophagic pathway of protein degradation might also be connected to cancer via some oncogenes and tumor-suppressor genes. Autophagy is partly controlled by the PI3K pathway, and constitutive activation of PI3K signaling is common in human cancer cells.[76] PI3K and its downstream effectors, Akt and TOR, might normally contribute to the suppression of autophagy, whereas PTEN (phosphatase and tensin homolog), a tumor suppressor that negatively regulates PI3K signaling, might normally promote autophagy. Furthermore, beclin 1 (BECN1) that interacts with PI3K and participates in the induction of autophagy in response to starvation is monoallelically deleted in a high percentage of human ovarian, breast, and prostate cancers.[77,78] Transfection of BECN1 into a transformed cell line can decrease its tumorigenic potential, and studies of mice that are deficient for this protein have shown that BECN1-mediated regulation of autophagy is required for normal mammalian development, and that animals with heterozygous deletions in Becn1 show a marked increase in the incidence of lymphomas and carcinomas of the lung and liver. Without autophagy, the natural turnover of a protein that acts as a positive regulator of cell growth might be blocked, promoting proliferation. Autophagy is also involved in removing damaged organelles and, therefore, in maintaining cellular homeostasis. Damage to mitochondria or sections of the endoplasmic reticulum might result in the production of endogenous cellular oxidants that increase the basal mutation rate. Removal of these damaged internal cellular structures by autophagy might therefore limit the genotoxic damage that is caused by oxidants. Conversely, reduced autophagy might increase oxidant stress and promote the accumulation of tumorigenic mutations.

Mitotic catastrophe is caused by aberrant mitosis and is associated with the formation of multinucleate, giant cells that contain uncondensed chromosomes. In normal somatic cells, the M phase of the cell cycle encompasses two processes: mitosis, in which sister chromatids are aligned and segregated into two daughter cells; and cytokinesis, in which the cytoplasm and its contents are partitioned into those cells. Mitosis is further subdivided into prophase, prometaphase, metaphase, anaphase, and telophase. The G2 checkpoint of the cell cycle is responsible for blocking mitosis when a cell has been hit by DNA damage. This activates a number of molecules that promote cellular activities such as cell-cycle arrest, DNA repair, or apoptosis, if the damage cannot be repaired. However, if the G2 checkpoint is defective, a cell can enter mitosis prematurely, before DNA replication is complete or DNA damage has been repaired. This aberrant mitosis causes the cell to undergo death by mitotic catastrophe. Activation of the G2 checkpoint begins with the detection of DNA damage by the sensory molecules ataxia telangiectasia mutated (ATM) and ATM- and Rad3-related (ATR). The further process involves activation of checkpoint kinase 2 (CHK2) and checkpoint kinase 1 (CHK1), and phosphorylation of CDC25C and several G2 checkpoint genes. The inhibition or inactivation of any of these G2-checkpoint genes results in the death of cells that have sustained DNA damage by mitotic catastrophe. Recent evidence suggests an important role of the cytoprotective protein survivin in checkpoint regulation.[79]

Defects in genes such as the polo-like kinase (PLK) family, the NIMA (for never in mitosis, gene A) family, the aurora kinase family, and a regulator of the spindle checkpoint called BUB that are required for mitotic catastrophe can also contribute to tumorigenesis.[80] Overexpression of aurora-A, which occurs in a wide range of human cancers, increases genetic instability, aneuploidy, and centrosomal aberrations. Furthermore, the overexpression of other mitotic kinases results in multinucleation and an increase in centrosome number.[81,82]

Cancer cells often have a defect in a particular cell-death pathway, but the cells can still die because of the redundancy of cell-death mechanisms. However, the nature of the cell-death defect ultimately affects the clinical outcome of treatment, depending on which mechanism is missing. At present it is not clear whether apoptosis, senescence, necrosis, autophagy, and mitotic catastrophe are entirely independent programs, whether these mechanisms overlap to some degree, or whether one mechanism may compensate for another that is inactivated by a tumorigenic mutation.

Factors influencing tumor growth

Since Virchow postulated that inflammation stimulates the progression of cancer, evidence has been found that immune-cell infiltration is a characteristic of malignant tumors. Tumor cells produce various cytokines and chemokines that attract macrophages, dendritic cells, mast cells, T cells, and hematopoietic progenitors. These stromal cells sometimes even outnumber cancer cells. Besides releasing mitogenic and survival factors, stimulating DNA damage, facilitating invasion by remodeling the extracellular