Fig. 1.1 Data from Center for Disease Control.

Fig. 1.2 Cancer is a clonal disease. Expansion of the original clone (dark gray) is followed by emergence of a new clone (black), which gradually replaces the original.

Fig. 1.3 A highly stylized potential "life history" of a cancer cell. Cancer cells are shown in grey (different shades, subclones); stromal cells are shown in vertical lines, vessels in dark grey.

Fig. 1.4 Causes of death. (Leading causes of death worldwide* up to 1997, expressed as millions of deaths per annum).*In eight defined regions of the world, including developed and developing areas. From Murray, C.J.L. and Lopez, A.D. (1997) Mortality by cause for eight regions of the world: global burden of disease study. *Lancet*, **349**: 1269–76.

Fig. 1.5 Processes contributing to regulation of tissue mass. Cell mass is determined by the balance of various cellular processes including at two extremes growth/replication and cell death.

Fig. 1.6 Processes contributing to cancer formation. The "hallmark" features of cancer shown appearing in a potential sequence. It should be noted that this does not imply that this is the actual sequence in which such features are acquired in any particular cancer.

Fig. 1.7 Cell of origin of cancer. Cancers probably originate most frequently in progenitor or stem cells, but could also arise from more differentiated cells that lose differentiation as part of the oncogenic process.

Fig. 1.8 Loss of heterozygosity through various genetic events.

Fig. 1.9 Linkage between signaling regulating replication, DNA damage, apoptosis, and growth arrest.

Fig. 1.10 The complexity of cellular information flow in cancer.

Fig. 1.11 Tumorigenesis ultimately results from disordered gene expression. Tumor cells arise through aberrant expression of genes and the proteins they encode. This may result from: mutations in the coding or noncoding regulatory regions of genes, which can be either inherited or acquired in somatic cells or even by major rearrangements of the chromosomes; epigenetic factors such as altered patterns of methylation and acetylation, which control the "accessibility" of genes for transcription. These events may in turn affect the stability and processing of RNA or proteins.

Fig. 2.1 Typical picture of SVCO in patient with lung cancer, showing dilated veins in neck and on chest wall.

Fig. 2.2 Patient with cerebral metastases from lung cancer, showing features of cranial nerve damage with bilateral ptosis.

Fig. 2.3 Patient undergoing bronchoscopy.

Fig. 2.4 Mammographic image of primary breast tumor.

Fig. 2.5 Primary breast tumor showing extensive local skin involvement.

Fig. 2.6 Breast cancer showing "*peau d'orange*" in left breast.

Fig. 2.7 Plain X-ray of right humerus showing lytic bone metastases.

Fig. 2.8 Barium enema showing malignant lesion at site of abnormal barium filling (arrowed).

Fig. 2.9 MRI image of pelvis showing large rectal tumor.

Fig. 2.10 Skin lesions in extensive malignant melanoma.

Fig. 2.11 Histological section of lymph node from patient with Hodgkin's disease showing typical large Reed–Sternberg cell.

Fig. 2.12 Enlarged lymph node in a patient with Burkitt's lymphoma.

Fig. 3.1 Cancer susceptibility is due to a combination of genetic and environmental factors. The cumulative effects of both genetic/epigenetic changes and exposure to environmental factors determine the likelihood of developing cancer. In this model, only in extreme cases will cancers arise entirely due to either genetic or environmental factors alone.

Fig. 3.2 The combination of genes and environment in the genesis of a cancer cell. This diagram describes a potential example of how inherited and acquired genetic alterations together with environmental factors may lead to tumorigenesis and cancer.

Fig. 3.3 Genetic variation may influence susceptibility to carcinogens. Variation in gene alleles encoding proteins responsible for uptake, transport, and metabolism of a carcinogen may all affect the likelihood that the carcinogen will cause DNA damage. Moreover, variation in DNA repair processes will also play a role.

Fig. 3.4 Multistage carcinogenesis, concept of initiation and promotion. Genetic changes induced by mutagens are irreversible but may be phenotypically occult until further events such as proliferation or loss of differentiation unmask them. The mutagen/carcinogen is the "tumor initiator," but other factors (tumor promoters) affect whether mutated cells proliferate and form tumors. Promoters can contribute to cancer formation but do not alter DNA. Promoters increase the frequency of tumor formation in tissues previously exposed to mutagen/tumor initiator. For example, skin papillomas form after exposure to carcinogens, but there may be considerable latency between the mutation in the stem cell pool and exposure to a tumor promoter that promotes proliferation, resulting in a visible lesion. This expanded clone will then itself be vulnerable for subsequent mutational events (hits) for tumor progression. Some evidence suggests that angiogenesis may accompany the growth of the tumor, or may arise through a distinct mutational event (angiogenic switch). Finally an invasive cancer forms with cells entering the circulation. However, it is generally believed that a further mutational event (such as inactivation of a "metastasis-suppressor" gene) is needed for colonies of cancer cells to establish in a distant location.

Fig. 3.5 Colorectal cancer as a model of multistage carcinogenesis – the adenoma–carcinoma sequence. In this cancer model sequential acquisition of mutations in various genes, shown in hatched boxes, is associated with initiation and progression of cancer. However, this does not mean that all colonic cancers arise in this sequence – activation of oncogene and inactivation of tumor-suppressor pathways is the key factor (alternative gene mutations could achieve the same net effect).

Fig. 3.6 DNA point mutations. For simplicity the small part of an mRNA transcribed from the normal or mutated gene is shown and the DNA (gene) itself is not shown. The DNA can be assumed to have a complementary sequence to the small section of mRNA shown. The mutated base is shown in dark grey.

Fig. 3.7 Breast cancer susceptibility (adapted from Balmain *et al*. 2003). Familial breast cancer accounts for around 5–10% of all breast cancers. However, known genes, such as *BRCA1* and *BRCA2* account for only 20% of the familial risk, so that most genetic factors contributing to breast cancer are unknown. These unknown genetic variants (probably at multiple different loci) interact with environmental factors in the pathoetiology of around 80% or more of breast cancers.

Fig. 4.1 The phases of the mammalian cell cycle.

Fig. 4.2 During the majority of the cell cycle (interphase), chromosomes are not visible under light microscopy. However, this is not the case during the nuclear division phase of mitosis, which is traditionally categorized, on the basis of early microscopy-based studies, into distinct observable stages that constitute the "chromosome cycle". Research over the last two decades has shown that the chromosome cycle is inextricably linked with the "cell cycle" (also referred to as the cyclin-dependent kinase – CDK cycle).

During mitosis the centrosomes duplicate, separate, and generate a microtubular spindle between them, thus providing a bipolar framework for the rest of mitosis. The chromosomes comprising two sister chromatids condense and become attached to microtubules arising from opposing spindle poles. Sister chromatids then separate and move apart to form two nuclei, which are ultimately separated by cytokinesis. Briefly, mitosis is divided into various phases based on early microscopy studies that correlate with distinct observable events.

During prophase the two centrosomes, each with a pair of centrioles, move to opposite poles of the cell, and the mitotic spindle forms and the chromosomes shorten and compact.

During prometaphase the nuclear envelope disappears and the kinetochore forms at each centromere. Spindle fibers attach to the kinetochores as well as to the arms of the chromosomes. For each dyad, one of the kinetochores is attached to one pole, the second(or sister) chromatid to the opposite pole.

At metaphase all dyads are arranged midway between the poles, called the metaphase plate. The chromosomes are at their most compact at this time.

At anaphase sister kinetochores separate (cohesions are broken down by separase, normally kept inactive by the chaperone securin; anaphase is initiated when the APC targets securin for proteasomal degradation). Each kinetochore moves to its respective pole along with its attached chromatid (chromosome).

During telophase a nuclear envelope reforms around each group of chromosomes, which re-adopt less compacted form.

Finally, during cytokinesis, the cell divides into two, through the action of actin filaments that constrict at the "waist" of the cell.

Fig. 4.3 Major checkpoints activated by DNA damage and mitotic defects in the cell cycle.

Fig. 4.4 Balancing of growth-regulating signals received in G_1, determines entry into S phase. Growth factor mitogens primarily act on the cell during the G_1 phase of the replication cycle.

Fig. 4.5 The cyclins and CDKs during the cell cycle – running the cell cycle "relay." The cell cycle resembles a relay race with a cyclin/CDK "runner" responsible for each of the four legs of the race. There are four classes of cyclins, each defined by the stage of the cell cycle at which they bind CDKs and function.

1. G_1/S-cyclins bind CDKs at the end of G_1 and commit the cell to DNA replication.
2. S-cyclins bind CDKs during S phase and are required for the initiation of DNA replication.
3. M-cyclins promote the events of mitosis.
4. G_1-cyclins help promote passage through Start or the restriction point in late G_1.

In G_1, the runners of the first leg (cyclin D–CDK4/6) are in the blocks waiting for the start gun (in G_0 they are stretching or milling around watching the long jump, some may have retired). The race starts in G_1 when the appropriate signal is received and the first baton is handed over by cyclin D – CDK4/6 to the S-phase cyclins after the restriction point. Once the race is on, each of the four runners drops out in sequence and another takes over for each of the four legs. Like in a race, the runners of the next leg sometimes start running before they get the baton and the previous runners take a while to stop running after the baton is handed over. If something minor goes wrong (a runner trips over) the race stalls but can restart once the problem is resolved. However, if something irretrievable happens (a baton is dropped) then the race is over.

Fig. 4.6 The G_1/S checkpoint. The G_1/S cell cycle checkpoint controls the passage of eukaryotic cells from the first "gap" phase (G_1) into the DNA synthesis phase (S). Two cell cycle kinases, CDK4/6-cyclin D and CDK2-cyclin E, and the transcription complex that includes RB and E2F, are key regulators of this checkpoint. In G_1, the RB–HDAC repressor complex binds to the E2F transcription factors, inhibiting transcription of S-phase genes. Phosphorylation of RB by CDK4/6 and CDK2 dissociates the RB–repressor complex, permitting transcription of S-phase genes (encoding proteins that amplify the G_1 to S-phase switch and that are required for DNA replication). Different stimuli exert checkpoint control including (i) TGF-β, (ii) DNA damage, (iii) contact inhibition, (iv) replicative senescence, (v) oncogenic stress, and (vi) growth factor withdrawal. The first five [(i) – (v)] act by inducing INK4 or KIP/CIP families of CKIs. TGF-β additionally inhibits the transcription of CDC25. Growth factor withdrawal activates GSK3β, which phosphorylates cyclin D, leading to its rapid ubiquitination and proteosomal degradation. Ubiquitination, nuclear export, and degradation are mechanisms often employed to rapidly reduce the concentration of cell cycle control proteins.

Fig. 4.7 The G_2/M checkpoint. The G_2/M DNA damage checkpoint prevents the cell from entering mitosis (M phase) if the genome is damaged. The CDK1 (CDC2)-cyclin B kinase is a key regulator of this transition. During G_2, CDK1 is maintained in an inactive state by the kinases WEE1 and MYT1. As M phase approaches, the phosphatase CDC25 is activated by the polo-kinase PLK1. CDC25 then activates CDK1, establishing a feedback amplification loop that efficiently drives the cell into mitosis. DNA damage activates the ATM/ATR kinases, initiating two parallel cascades that inactivate CDK1-cyclin B. The first cascade rapidly inhibits progression into mitosis: the CHK kinases phosphorylate and inactivate CDC25, which can no longer activate CDK1. Phosphorylation of p53 dissociates it from MDM2, activating its DNA binding activity. Acetylation by p300/PCAF further activates its transcriptional activity. The genes that are activated by p53 encode proteins including 14-3-3s, which bind phosphorylated CDK1-cyclin B promoting nuclear export; GADD45, which apparently binds to and dissociates the CDK1-cyclin B kinase; and $p21^{CIP1}$, an inhibitor of a subset of the CDKs including CDK1.

Fig. 4.8 The spindle checkpoint. A schematic of the spindle checkpoint pathway – in the left panel incorrectly attached kinetochores recruit BUB and MAD proteins, which form an inhibitory complex that catalyzes inactivation of the anaphase-promoting complex (APC/C) through MAD2 binding to CDC20. In the right panel, correct attachment and "tension" favors release of the checkpoint proteins from the kinetochore, releasing inhibition of APC/C allowing anaphase. This allows APC/C to ubiquitinate securin, which is then targeted for degradation by the proteasome. The APC/C also regulates degradation of B cyclins for mitotic exit. In mammalian cells securin at sites other than the centromere is removed by action of polo-like kinase during prophase.

Fig. 4.9 The two families of CKI proteins. The INK4 family of CKI ($p15^{INK4b}$, $p16^{INK4a}$, $p18^{INK4c}$, and $p19^{ARF}$) inhibit cyclin–CDK action by binding to the CDK, whereas CIP/KIP family members ($p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$) bind to the cyclin component of the cyclin–CDK complex.

Fig. 4.10 DNA synthesis during S phase. DNA is synthesized from 5′ to 3′ by the action of DNA polymerases.

Fig. 5.1 GFs act particularly at the G_1 phase of the cell cycle.

Fig. 5.2 Growth-regulatory factors derive from local and circulating sources.

Fig. 5.3 Cell division depends on the balance of different growth-regulating signals. Ultimately, the decision whether a cell lives or dies, replicates or does not, is dependent on the balance of positive and negative signals received.

Fig. 5.4 Main signaling pathways for mitogens. Mitogenic factors largely, but not exclusively, act via RTK to activate in particular the MAPK cascade.

Fig. 5.5 Positive and negative growth regulators determine G_1/S transition and cell cycling.

Fig. 5.6 Simple schematic of RTK signal transduction. Dimerization of RTK is triggered by ligand binding and activates the receptor. Activation involves tyrosine autophosphorylation and recruitment of various adapter proteins which then activate downstream signaling.

Fig. 5.7 Restraints on cell replication and motility may be temporarily released. During injury to tissues, cells may be allowed to replicate and migrate, but this is transient.

Fig. 5.8 Cooperation between oncogenic signaling pathways that regulate cell replication, and apoptosis.

Fig. 5.9 Tissue mass homeostasis.

Fig. 5.10 Growth factors may act via different routes. Secreted growth factors may act locally on the same cell (autocrine), or on neighboring cells (paracrine), or, at a distance (endocrine).

Fig. 5.11 Signaling through membrane-bound GF receptors. Positive and negative signals are integrated at various levels before a decision on whether to respond or not is completed.

Fig. 5.12 Signaling through nuclear receptors. Some hormones may act as ligands for nuclear receptors that may then act as transcription factors.

Fig. 5.13 Dimerization of ligand-bound RTK activates intrinsic TK activity. RTK are activated by ligand binding, which induces conformational changes, and receptor dimerization that activates intrinsic TK activity (panel 1). RTK can also become activated by viruses (panel 2) or by mutations (panel 3)

Fig. 5.14 Activation of RAS by RTK.

Fig. 5.15 Major growth-regulating signaling pathways activated by RTK. Amongst others, RTK can activate three major signaling pathways, RAS-RAF-MAPK; JAK-STAT and PI3K–AKT, all of which eventually signal to the nucleus and regulate gene expression. The latter is shown in greater detail as the other pathways are well covered in chapter 6. Some key tumor suppressors regulated or regulating this pathway are shown in bold.

Fig. 5.16 RAS–MAPK, a model growth factor-receptor-activated signaling pathway.

Fig. 5.17 Lipid signaling from receptor tyrosine kinases (such as IGF-1R, HER2/Neu, VEGF-R, PDGF-R): the PI3K and DAG – PKC pathways. RTK become activated by tyrosine autophosphorylation. Activated RTK associate with the p85 SH2 domain containing subunit, which recruits and activates the catalytic domain of PI3K. Activated PI3K in turn phosphorylates membrane inositol phospholipids in the 3′ position, which can then act as docking sites for proteins with pleckstrin homology (PH) domains, such as phospholipase cγ (PLCγ), PDK1, and AKT. PLCγ activates protein kinase C (PKC), important for transducing mitogenic signals, via diacylglycerol (DAG). The serine/threonine kinase AKT is activated by phosphorylation by PDK1/PIP3. AKT regulates phosphorylation of several downstream effectors, such as NF-κ B (NF-κB), mTOR, FOX01, BAD, glycogen synthase kinase (GSK-3), and MDM-2, which in turn influence cell growth, proliferation, protection from proapoptotic stimuli, and stimulation of neo-angiogenesis. AKT activates mTOR by phosphorylating the tuberous sclerosis 2 protein (TSC2); the resultant complex of TSC1/TSC2 is inactivated (loss of GTPase activity for RHEB), thus increasing active RHEB–GTP levels, which activate mTOR, leading to cell growth and replication. GSK-3 is a constitutively active proline-directed serine/threonine kinase that may induce apoptosis through multiple pathways. AKT can inactivate GSK-3 via phosphorylation thus reducing apoptosis.

Fig. 5.18 Growth factor activation by matrix metalloproteinases. Some growth factors are inactive whilst bound to extracellular matrix, but can be released by activity of matrix-degrading enzymes such as the matrix metalloproteinases.

Fig. 5.19 The Wnt signaling pathway. Binding of Wnt ligands to Frizzled allows the disassembly of the "destructosome" (comprising Axin, APC, and GSK3β). Specifically, Wnt signaling activates dishevelled, dishevelled binds to axin, excluding GSK3; thus β-catenin is able to avoid GSK3-mediated phosphorylation and thereby avoid proteasomal degradation; β-catenin accumulates in the nucleus and activates TCF/LEF transcription factors, which in turn activate growth-regulatory genes, such as c-MYC and cyclin D.

Fig. 6.1 Representative oncogene products. In healthy cells, the normal nonmutated counterpart, proto-oncogenes, encode proteins that in general promote cell proliferation or cell survival but which are under tight regulation to avoid excessive proliferation, or indeed the survival of a cell that has sustained DNA damage and would normally be got rid of. Oncogenes include a diverse range of genes encoding proteins involved in the regulation of cell division, differentiation, and death/survival. The majority of oncogenes fall into five functional classes: transcription factors, growth factors, receptors, signal transducers, and survival proteins. Some key examples of representative oncogene protein products are shown here.

Fig. 6.2 Conversion of proto-oncogenes to oncogenes. A proto-oncogene can be converted to an oncogene in a number of ways. These include point mutations in a single gene (e.g. *RAS*) that can either affect the coding region of the gene resulting in the formation of an abnormal oncoprotein with enhanced stability or activity, or may affect regulatory elements resulting in enhanced or deregulated expression. Chromosomal translocations or rearrangements can lead to overexpression of an oncoprotein. For example, in Burkitt's lymphoma, the proto-oncogene *c-MYC* on chromosome 8 is translocated to one of the three chromosomes containing the genes that encode antibody molecules: immunoglobulin heavy chain locus (chromosome 14) or one of the light chain loci (chromosome 2 or 22). *c-MYC* now finds itself in a region of vigorous gene transcription, leading to overproduction of the c-MYC protein. Gene amplification can lead to overexpression of the oncogene. Lastly, the fusion of one protein to another might lead to its constitutive activity. For example, fusion of the promyelocytic leukemia (PML) protein to the retinoic acid receptor-alpha (RARα) generates the transforming protein of acute promyelocytic leukemias.

Fig. 6.3 Oncogene collaboration *in vitro*. Transfection of NIH 3T3 fibroblasts with DNA from tumors or tumor cell lines results in some cells becoming transferred as demonstrated by loss of normal contact inhibition and formation of foci (colonies) of cells piled up rather than in a monolayer. This same result could subsequently be achieved by transfecting NIH 3T3 cells with oncogenic *RAS*. In contrast, transfection of a "more normal" rat embryo fibroblast with any single oncogene (either *RAS* or *MYC* alone) did not result in transfomation. However, if both oncogenic *RAS* and *MYC* were transfected into rat embryo fibroblasts then foci did form – oncogene cooperation. By implication NIH 3T3 fibroblasts have already undergone mutations such as those likely to occur in multistep tumorigenesis, thus enabling them to be more readily transformed in cell culture.

Fig. 6.4 c-MYC promotes cell death by apoptosis (*in vitro*). In the early 1990s, several laboratories made an intriguing discovery: oncoproteins such as c-MYC and the adenovirus E1A – both potent inducers of cell proliferation – were shown to possess apoptotic activity. Ectopic expression of c-MYC in fibroblasts that were cultured in the absence (or limited supply) of survival factors [e.g. factors that are present in fetal calf serum, such as the extracellular molecule insulin growth factor 1 (IGF-1), which mediates cell survival via its receptor] led to apoptosis, with the eventual loss of the entire cell population. Although interpreted by some as a conflict of growth signals, oncogenes activate apoptosis if the proliferative pathway is blocked in some way. The most widely held view of oncoprotein-induced apoptosis is that the induction of cell cycle entry sensitizes the cell to apoptosis, in other words, cell proliferative and apoptotic pathways are coupled. However, the apoptotic pathway is suppressed as long as appropriate survival factors deliver antiapoptotic signals. In this scenario, the predominant outcome of these contradictory processes will depend on the availability of survival factors.

Fig. 6.5 Functional domains of human c-MYC protein. (a) The C-terminal domain (CTD) of human c-MYC protein harbors the basic (b) helix–loop–helix leucine zipper (bHLH-LZ) motif for dimerization with its partner, Max, and subsequent DNA binding of c-MYC–Max heterodimers. The N-terminal domain (NTD) harbors conserved "c-MYC boxes" I and II (MBI and MBII) essential for transactivation of *c-MYC* target genes. Recently the MBIII situated in the central region has been found to be important for negatively regulating the apoptotic response. (b) Some major c-MYC-interacting proteins that may or may not bind simultaneously to c-MYC. These include coactivator TRRAP, part of a complex possessing histone acetyltransferase (HAT) activity, which interacts with MBII region and mediates chromatin remodeling. TIP48 and TIP49 proteins interact with the NTD of c-MYC and are implicated in chromatin remodeling due to their ATP-hydrolyzing and helicase activities. Proteins involved in transcriptional regulation, such as Miz-1, interact with the CTD of c-MYC, whereas SP1 interacts with the central region of c-MYC. NLS; nuclear localization signal.

Fig. 6.6 c-MYC induces cell cycle entry through activation and repression of target genes. (a) MYC – MAX heterodimers activate target genes, *CCND2* (cyclin D2) and cyclin-dependent kinase 4 (*Cdk4*), which leads to sequestration of CDK inhibitor KIP1 $p27^{KIP1}$ in cyclin D2 – Cdk4 complexes. Subsequent degradation of KIP1 involves two further MYC target genes, *CUL-1* and *CKS*. In so doing, KIP1 is not available to bind to and inhibit cyclin E – CDK2 complexes, thereby allowing cyclin E – Cdk2 to be phosphorylated by cyclin-activating kinase (CAK). Activation of some genes by MYC – MAX involves displacement of the putative tumor suppressor MNT from target genes. (b) MYC – MAX heterodimers repress CDK inhibitors, $p15^{INK4B}$ and WAF1 (p21), which are involved in cell cycle arrest. By interacting with transcription factors, MIZ-1 (and/or Sp1), MYC – MAX prevents transactivation of INK4B (CDKN2B) and WAF1 (CDKN1A).

Fig. 6.7 MYC – Max and Mad – Max heterodimers regulate gene activation through chromatin remodeling. (a) MYC – MAX heterodimers binds to an E-box sequence (CACGTG) near the promoter of a *c-MYC* target gene. Coactivator TRRAP (transformation/transcription domain-associated protein), a component of a complex that contains HAT activity, is then recruited to the MYC box II (MBII) domain of c-MYC and acetylates (Ac) nucleosomal histone H4 at the E-box and adjacent regions. Nucleosomal acetylation alters chromatin structure, allowing accessibility of MYC – MAX transcriptional-activator complexes to target DNA, resulting in expression of the target gene. (b) Induction of Mad during terminal differentiation results in the Mad – Max heterodimer binding to an E-box of a *c-MYC* target gene. Corepressor SIN3 and histone deacetylases (HDACs) are then recruited to MAD, resulting in local nucleosomal histone deacetylation and repression of target-gene expression.

Fig. 6.8 Cooperation between RAS and MYC. (a) Activation of mitogenic proteins, such as c-MYC and RAS, triggers not only pathways that lead to cell cycling but also those that promote cell death (apoptosis) and growth arrest/senescence, respectively. Deregulated activation of c-MYC alone may preferentially lead to apoptosis rather than cell division, while activated RAS alone may lead to cellular senescence – these outcomes serve to protect the organism from cancer-inherent tumor-suppressor activity. However, when RAS and MYC are both activated, these "in-built" tumor suppressor activities are lost: RAS suppresses MYC-induced apoptosis and MYC suppresses RAS-induced growth arrest. It is such cooperation between oncogenes that promotes tumor development. (b) Activation of RAS and MYC results in potential engagement of both replication and growth but also of apoptosis and possibly senescence. If either MYC or RAS levels are excessive (as might occur during oncogenesis) or other proapoptotic signals are received then the balance may be tipped away from replication. RAS can promote senescence through either $p16^{INK4a}$ or ARF, which activate RB or p53 pathways respectively, while MYC can inhibit growth arrest/senescence by inhibiting $p21^{CIP1}$ and inducing TERT (telomerese reverse transcriptase). RAS may also activate $p21^{CIP1}$ via RAF activation. Although MYC may activate the apoptotic pathway (e.g. via ARF), RAS is able to suppress apoptosis by activating the PI3K pathway and, subsequently, AKT. It can readily be appreciated how oncogenic MYC and RAS may conspire in oncogenesis. The combination of RAS and MYC acting together provides a potential means of avoiding apoptotic and senescence mechanisms activated by either acting individually. Moreover, it can also be appreciated how inactivating mutations in RB or p53 (or their pathways involving $p19^{Arf}$, $p16^{INK4a}$, $p21^{CIP1}$ etc.) may contribute to tumorigenesis by enabling the cancer cell to avoid either senescence, or apoptosis, or both.

Fig. 6.9 (a) A system has been developed allowing the ectopic activation of c-MYC in various tissues *in vivo*. A transgene encoding a chimeric protein (c-MYC fused to the ligand-binding domain of a modified estrogen receptor ER^{TAM}, which now responds only to the synthetic SERM, 4-hydroxy tamoxifen- 4-OHT) is placed under a tissue-specific promoter directing expression to a predetermined tissue (Involucrin – Inv – for suprabasl keratinocytes or Insulin – pIns – for pancreatic β cells). Activation of c-MYC is achieved by administration of 4-OHT, which binds to the ER^{TAM}, thus displacing heat shock proteins that otherwise hold the protein in an inactive state. (b) Normal adult wild-type skin (or skin in transgenic mice prior to c-MYC activation) is shown in the left-hand panel. In general the epidermis (E) is only two cells thick (more in man), comprising a basal layer (containing stem cell precursors) and a suprabasal layer containing differentiating skin keratinocytes. Underlying the epidermis is the dermis. The upper panels are hematoxylin-and-eosin stained to show structural features. The lower panels show cell nuclei in blue (DAPI) and the nuclei of replicating cells in green (FITC), as they are stained with an antibody to a cell cycle marker – Ki67. The right-hand panels show the effects of activating c-MYC for 2 weeks. The epidermis is greatly expanded and now contains large numbers of replicating cells. Not only is the epidermis expanded but there are now large keratotic spires (keratinized dead or dying cells that remain nucleated – normally cells lose their nuclei at the surface and are then shed) forming papillomas (P). These papillomas become vascularized by angiogenesis (black arrow). Although not shown here, deactivating c-MYC results in complete reversal of this aberrant phenotype and restoration of normal appearing skin. (Please see our website for the color version of this figure).

Fig. 6.10 The oncogenic potential of c-MYC is exposed in pancreatic β cells when apoptosis is blocked. (a) Activation of c-MYC in β cells of the pancreatic islets results in cell cycle entry (Ki67 positive cells – stained brown), but then also results in β-cell apoptosis (TUNEL positive cells – stained brown). In fact, the net effect is almost complete ablation of the β-cell population within 10 days, resulting in diabetes. In this tissue, unlike in skin, the net effect of deregulated c-MYC expression is apoptosis, and avoidance of neoplasia. If, however, c-MYC apoptosis of β cells is prevented by concurrent overexpression of the antiapoptotic protein BCL-x_L, then the devastating oncogenic properties of unapposed c-MYC action are unmasked – culminating in relentless replication, avoidance of apoptosis, loss of differentiation, loss of cell – cell contacts, angiogenesis, and invasion – the "hallmark features" of cancer. (b) This intrinsic "tumor suppressor" function of c-MYC is illustrated schematically. (Please see our website for the color version of this figure).

Fig. 6.11 RAS activation depends on regulation of GTP/GDP exchange. (a) In a healthy cell, RAS proteins regulate diverse cellular processes by cycling between biologically active GTP- and inactive GDP-bound conformations. In the active state, RAS proteins are bound to GTP and are thus able to engage downstream effectors that activate signaling pathways controlling several aspects of cell behavior. When bound to GDP, RAS proteins are inactive and so fail to interact with these effectors. The conversion of bound GTP to GDP, and vice versa, is catalyzed within the cell: the nucleotide exchange by guanine nucleotide exchange factors (GEFs), such as Sos, and the nucleotide hydrolysis by GTPase activating proteins (GAPs), such as NF1. It is the balance between these proteins that will ultimately determine the activation state of RAS and its downstream pathways. (b)Activation of RAS in tumors: mutations in RAS can prevent the intrinsic GTPase activity and impede exchange of GTP for GDP, thus favoring the active conformation. A similar outcome can result from loss of the NF1 tumor-suppressor protein, which normally amplifies the intrinsic GTPase activity of RAS. Although growth factor stimulation will still play some role in phenotype, these alterations can result in constitutive RAS activation.

Fig. 6.12 Lipid modification of RAS. RAS activation (exchange of bound GDP for GTP) requires localization of RAS-GDP to the inner cell membrane, presumably because in this location it is more readily available for interaction with growth factor receptor associated proteins. Usually this is achieved by addition of enzymatic addition of prenyl groups, primarily farnesyls. Though if farnesyl transferase is inhibited, at least for Ki-RAS, then these can be replaced effectively by geranylgeranyl groups, which appear to serve the same or similar purpose.

Fig. 6.13 Signaling pathways regulated by RAS. Active GTP-bound RAS will interact with several families of effector proteins, with the most important shown. RAF protein kinases initiate the MAPK cascade, which leads to ERK activation. ERK has numerous substrates including ETS family transcription factors such as ELK1 that regulate cell cycle progression. Phosphoinositide 3-kinases (PI3Ks) generate lipid messengers, such as phosphatidylinositol-3,4,5-trisphosphate, which activate the kinase AKT/PKB, involved in survival. RALGDS proteins are GEFs for RAL, an RAS-related protein. Downstream targets include forkhead transcription factors. Phospholipase C (PLC) catalyzes the hydrolysis of phosphatidylinositol-4,5-bisphosphate to diacylglycerol and inositol trisphosphate, resulting in protein kinase C (PKC) activation and calcium mobilization from intracellular stores.

Fig. 6.14 The RAS – RAF signaling pathway. Receptor dimerization on ligand binding induces tyrosine autophosphorylation of the RTK, binding of GRB2 to the receptor via SH2 domain, and translocation of the GEF, SOS, to the cell membrane. SOS in turn promotes RAS activation by enhancing GDP – GTP exchange. A third adaptor protein SHC (also carrying an SH2 domain) may be phosphorylated by growth factor binding and can recruit the GRB2/SOS complex. Activated RAS by either of these two overlapping pathways in turn phosphorylates the serine/threonine kinase RAF, in part by a conformational effect and replacement of 14-3-3 protein. RAF phosphorylates and activates MAP kinase/ERK kinase (MEK). MEK then activates ERK, which can then translocate to the nucleus and activate transcription factors, such as ETS family, c-FOS, and c-JUN (which together form the AP1 transcription factor), c-MYC, and others.

Fig. 6.15 Oncogenic RAS – pathways to survival or death. Tumor development and maintenance depends on the ability of a cell to avoid apoptosis. Although RAS is known to promote cell survival by preventing apoptosis, in some circumstances, it has been shown that RAS can also mediate proapoptotic signals. The ultimate outcome of these contradictory signals depends greatly on the cell type and context. RAS can promote cell survival through a number of signaling pathways. A key pathway that predominantly mediates survival is the PI3K signaling cascade, which activates the serine/threonine (ST) kinase AKT. In turn, AKT phosphorylates a number of substrates including Bad – a proapoptotic member of the BCL-2 family. AKT phosphorylation of Bad causes it to bind preferentially to 14-3-3 proteins in an inactive complex, thereby preventing it from sequestering and inactivating the antiapoptotic proteins BCL-2 and BCL-x_L. However, AKT may also phosphorylate the Thr 58 residue on c-MYC protein, leading to its increased stability (as discussed in the main text); c-MYC can promote apoptosis. RAS signaling through PI3K can also mediate survival by downregulating the proapoptotic BCL-2 family member Bak, and by preventing downregulation of the antiapoptotic protein BCL-x_L. In addition, PI3K can promote survival through the transcription factor NFκB. RAS signaling through RAFvERK can be either anti- or proapoptotic, depending on the circumstances. This pathway can lead to stabilization of c-MYC through phosphorylation of its Ser 62 residue (proapoptotic) or through phosphorylation of Bad as described above, which promotes survival. Lastly, although in many cases oncogenic RAS can provoke a response from p53 designed to cause cell cycle arrest or apoptosis, to complicate matters it can also suppress p53 by inducing its degradation via Mdm2 in a RAF-dependent manner. RAF may also contribute to the ability of oncogenic RAS to provide a prosurvival function by downregulating transcription of Par-4, a proapoptotic transcriptional repressor. RAF and PI3K signaling may also converge downstream of oncogenic RAS to prevent apoptosis.

Fig. 6.16 RHO GTPases mediate a diverse range of cellular effects. The high incidence of overexpression of some members of the RHO family of GTPases in human tumors suggests that these proteins are involved in cancer onset, and that they are potential candidates for a therapeutic intervention. RHO GTPases, the most widely studied of which are RHOA/B, RAC1/2, and CDC42, mediate a diverse range of cellular effects, such as proliferation, motility, and adhesiveness, although the precise mechanisms by which RHO GTPases participate in carcinogenesis are still not fully understood. However, it is becoming more evident that the specific role of RHO overexpression in tumor initiation, progression, and metastasis may be linked to the activation of specific signaling pathways that result in transcriptional regulation.

Fig. 6.17 RHO promotes tumor cell properties through activation of specific transcription factors. (a) RHO GTPases (RHOA, RAC1, and CDC42) can activate Stat3 (signal transducer and activator of transcription 3) transcription factor by indirectly inducing its phosphorylation (via JNK2). It has recently been shown that RHOA (bound to the effector protein Rock) signals through Stat3 for transformation, by inducing proliferation and anchorage-independent cell growth – hallmarks of transformed cells. Active Stat3 may promote cell proliferation by inducing the expression of c-MYC or cyclin D1 Although RAC1 and CDC42 also activate Stat3, it is not yet known whether these GTPases are able to induce transformation via Stat3. (b) RHOA, RAC1, and CDC42 proteins also activate Stat5a. RHOA activates Stat5a by indirect phosphorylation, causing cells to become more motile and inducing epithelial-to-mesenchymal transition (EMT), features associated with tumor cell behavior. Stat5a may mediate such cellular effects by upregulating the expression of vimentin that is associated with EMT, while downregulating E-cadherin expression with loss of adherens junctions (these mediate cell–cell adhesion). RHOA can also induce EMT by activating another transcription factor, SRF. (c) RHOA, RAC1, and CDC42 proteins induce activity of the transcription factor, NFκB. NFκB can promote cell proliferation, cell survival, invasion and motility of cancer cells, presumably through expression of its target genes. One target gene is the urokinase plasminogen receptor gene (*uPAR*), whose expression results in enhanced invasiveness due to degradation of the extracellular matrix. Other target genes include the cell cycle protein cyclin *D1* and the proinflammatory gene cyclooxygenase 2 (*COX-2*) that is associated with cancer. Finally, the RHOA – Rock pathway can activate c-MYC, leading to repression of the antiangiogenic factor, thrombospondin-1 (Tsp-1), which in turn allows proper angiogenesis to take place within the tumor.

Fig. 6.18 Regulation and activation of c-SRC. (a) The functional domains of human c-SRC: human c-SRC protein contains four SRC homology (SH domains) comprising the SH1 kinase domain, which contains the autophosphorylation site (Tyr419); the SH2 domain, which interacts with the negative-regulatory Tyr530 and binds to the platelet-derived growth factor receptor (PDGFR); the SH3 domain, which enhances interactions with the kinase domain; and the SH4 domain, which contains the myristoylation site required for membrane localization. (b) Activation of c-SRC: when the C-terminal tyrosine Tyr530 is phosphorylated, SRC is inactive and assumes a "closed" conformation as a result of interactions between the C-terminus and the SH2 domain, and between the kinase domain and the SH3 domain. This closed configuration prevents substrate interaction, and the inactive c-SRC protein resides at a perinuclear site. Inactivation of c-SRC by phosphorylation is performed by CSK (c-SRC tyrosine kinase). Following binding of PDGF ligand to its receptor or after integrin engagement, SRC becomes active and translocates to the cell membrane. This activity is the result of dephosphorylation of Tyr530 that leads to an "open" conformation of the protein, thus allowing substrate interaction. Protein tyrosine phosphatases (PTP) have been shown to dephosphorylate the Tyr530 of c-SRC.

Fig. 6.19 Oncogenic SRC can affect cell behavior in various ways. Oncogenic forms of SRC can activate signaling pathways to alter cell structure, in particular the actin cytoskeleton and the adhesion networks that control cell motility and invasion. In addition, SRC can transmit signals that regulate cell proliferation and survival, as well as angiogenesis. Details of these signaling pathways are described in Fig. 6.20.

Fig. 6.20 Signaling pathways activated by oncogenic SRC to promote tumor cell behavior. The two principal subcellular structures that regulate adhesion, invasion, and motility in normal healthy cells – focal adhesions and adherens junction – are regulated by *c-SRC*. Focal adhesions provide the structural and mechanical properties that are necessary for cell–matrix attachment and form at the sites where integrins link the actin cytoskeleton to extracellular matrix (ECM) proteins. Adherens junctions enable neighboring cells to adhere to each other: cell–cell attachment. Activated SRC plays a key role in disassembly of focal adhesions that promotes detachment from the ECM, a property that is acquired by cancer cells. It does this through its binding and activation of FAK (focal adhesion kinase): activated FAK phosphorylates p190 RhoGap that in turn inhibits RhoA leading to focal adhesion disruption. SRC can also destabilize focal adhesions through its tyrosine phosphorylation of R-RAS protein. Activated SRC can also promote the release of cells from each other by disrupting adherens junctions by inducing tyrosine phosphorylation and ubiquitylation of the E-cadherin complex. As a result, cadherin molecules are internalized. Phosphorylation of FAK by *c-SRC* is also required for the disruption of E-cadherin cell–cell adhesion. SRC might also promote invasion by regulating MMPs, enzymes that can break down extracellular matrix proteins and thus promote tumor cell invasion and angiogenesis. SRC activation of FAK stimulates the JNK pathway, ultimately leading to the expression of MMP2 and MMP9. Lastly, SRC proteins can promote angiogenesis through the expression of VEGF, by activating the transcription factor STAT3.

Fig. 6.21 Tumor regression following deactivation of initiating oncogenic lesion. Various transgenic mouse models of tumorigenesis have shown that subsequent deactivation of the initial oncogenic lesion leads to rapid and complete regression of most neoplastic lesions. Mechanisms of tumor regression include tumor cell apoptosis and/or differentiation and re-establishment of cell – cell contacts. Onset of vasculature collapse within tumor masses occurs rapidly and may also contribute to tumor cell apoptosis. In some cases, a subset of tumors escape reversal ("escapers") and thus are no longer maintained by the initiating oncogenic lesion. In these cases, it is likely that additional oncogenic lesions have occurred.

Fig. 7.1 The "two hit" hypothesis.

Fig. 7.2 Linear structures of the Rb family: "pocket proteins." Schematic representation of the three members of the Rb family: pRb, p107, and p130. Note the conservation of many of the domains, including the A–B pocket and the differences in length of the spacer, the N- and C-termini.

Fig. 7.3 Rb family of proteins – activation and G_1 progression. In G_0 and early G_1, pRb, p107 and p130 are underphosphorylated and in complex with E2F-4, E2F-5 and the DP1 and 2 proteins, repressing transcription. pRb also sequesters E2F-1, 2, and 3a/DP complexes without contacting DNA. In G_1, cyclin D/Cdk4–6 and cyclin E/Cdk2 holoenzymes phosphorylate pRb proteins and relieve their repressive function allowing E2Fs to induce the transcription of E2F-responsive genes required for S phase entry.

Fig. 7.4 Rb family of proteins – activation and repression of gene expression.
Gene repression: (a) pRb, p107, and p130 repress transcription (OFF) by contacting DNA via E2F-4, E2F-5, and DP complexes and recruiting histone deacetylase (HDAC). Histones are not acetylated and the chromatin is condensed. (b) Repression can be mediated by E2F-3b bound to as yet unidentified repressor proteins. (c) pRb sequesters E2F-1, 2, and 3a and their DP partners and inhibits their function without contacting DNA.
Gene activation: Phosphorylation of pRb releases E2F-1, 2 and 3a, and DP partners, which recruit histone acetylase and induce the transcription of E2F-responsive genes. Histones are acetylated; the chromatin is opened allowing gene transcription (ON).

Fig. 7.5 The Rb signaling pathway. In G_0, Cdk4 is bound to the chaperone protein Hsp90, and to Cdc37 while cyclin E/Cdk2 is bound to $p27^{Kip1}$. Both kinases are inactive. In G_1, upon mitogen activation, receptors activate the Ras/MAP kinase pathway that regulates cyclin D transcription and the release of Cdk4 from Hsp90 and Cdc37. Cdk4 kinase activity is induced by binding to D-type cyclins, its phosphorylation by the cyclin-dependent kinase (CAK) and $p27^{Kip1}$, which serves as an assembly factor. The active cyclin D/Cdk4–6 holoenzyme initiates the phosphorylation of Rb. Binding of $p27^{Kip1}$ to increased levels of cyclin D/Cdk4–6 complexes relieve the inhibition of cyclin E/Cdk2 complexes that complete the phosphorylation of Rb and phosphorylate $p27^{Kip1}$, which is then degraded by the proteasome machinery. Phosphorylation of Rb releases E2F1, 2 and 3a, which transactivate the expression of E2F-responsive genes, including cyclin E, cyclin A, and E2F-1 and genes required for the initiation of DNA replication (S phase).

Fig. 7.6 Cell cycle exit – quiescence and senescence. In mid-G_1 and in response to antiproliferative signals, Ink4 proteins are induced. Ink4 proteins bind to Cdk4–6 and free cyclin D, which is rapidly degraded. $p27^{Kip1}$ is reassorted to cyclin E/Cdk2 complexes to inhibit their kinase activity. Rb phosphorylation is inhibited, cells can no longer enter S phase and cells are arrested in G_1.

Fig. 7.7 Structure of p53 family of proteins. Schematic linear structure of p53, p63, and p73. Note that all three proteins share common domains but contain negative regulatory domains (NRD) of different lengths.

Fig. 7.8 p53 activation and function. The transcription factor p53 is activated by phosphorylation in response to hyperproliferative signals and genotoxic stress. This induces an accumulation of p53 in the nucleus and the transcription of multiple p53 target genes that induce growth arrest, apoptosis, DNA repair, or a block in angiogenesis.

Fig. 7.9 *p53* mutations in human cancer. Mutations in the p53 gene have been found throughout the entire coding sequence but with increased frequency in the DNA-binding domain. Mutations affect p53 transcriptional activity. Reproduced from the IARC database (www.iarc.fr/p53), R9 July 2004. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 Database: new online mutation analysis and recommendations to users. *Hum Mutat* 2002 Jun: 19(6): 607–14.

Fig. 7.10 The *INK4A/ARF* genomic locus. Schematic representation of genomic organization of the genes that are encoded by the locus. $p16^{INK4a}$ is encoded by three exons, exon 1α, exon 2, and exon 3. Exon 2 also encodes another protein, but in an alternative reading frame, i.e., $p19^{Arf}$ ($p14^{ARF}$ in humans). *ARF* transcription is initiated from exon 1β. Another protein, $p15^{INK4b}$, is encoded by two exons that are genetically linked to the *INK4a/ARF* locus.

Fig. 7.11 The *INK4a/ARF* locus encodes two tumor suppressors. The same exon 2 encodes two tumor suppressor proteins, $p14^{ARF}$ ($p19^{Arf}$ in the mouse) and $p16^{INK4a}$. Whereas the tumor suppressor $p14^{ARF}$ activates p53 by binding to and sequestering its negative regulator Mdm2, the tumor suppressor $p16^{INK4a}$ binds to and inhibits cyclin D/Cdk4 activity and pRb phosphorylation. Expression of $p14^{ARF}$ induces G_1 and G_2 arrest while enforced expression of $p16^{INK4a}$ induces G_1 arrest. Thus, remarkably, the *INK4a/ARF* locus encodes two tumor suppressors that regulate two of the most important tumor suppressors in human cancers.

Fig. 7.12 ARF activates p53 by sequestration of Mdm2. Mdm2 is a negative regulator of p53 that induces its degradation. In response to hyperproliferative signals due to oncogene activation, including overexpression of Myc or expression of a constitutively active RasV12 protein, ARF is induced, it binds to Mdm2, which results in the accumulation and activation of p53 leading to cell cycle arrest or apoptosis.

Fig. 7.13 The Arf/Mdm2/p53 signaling pathway. Transcription of the tumor suppressor $p19^{Arf}$ is induced by Myc, E2F and others (in green box). *Arf* expression is actively repressed by repressor protein complexes, including Bmi1 (in red box). ARF activates p53 by sequestration and inhibition of Mdm2 to induce cell cycle arrest or apoptosis. $p19^{Arf}$ also induces growth arrest in a p53-independent manner by inducing many antiproliferative genes (including the Btg proteins), which in concert induce cell cycle arrest.

Fig. 7.14 Coupling of pRb and p53 signaling pathways. ARF expression is induced in response to hyperproliferative signals. Ras induces cyclin D transcription. Cyclin D forms active complexes with Cdk4–6, which phosphorylate pRb and release E2Fs. E2Fs and Myc induce ARF expression. ARF activates p53 by sequestering Mdm2. p53 in turn activates the transcription of p53-responsive genes responsible for growth arrest, apoptosis (cell death), or DNA repair. Adapted from Sherr (1998). Reproduced by permission of Cold Spring Harbor Laboratory Press.

Fig. 7.15 The PI3K–AKT survival pathway – PTEN phosphatase. Growth factors by binding to their respective receptors (GFR) activate the phosphatidylinositol-3 kinase (PI3K), which converts phosphatidyl inositol 4,5 biphosphate (PIP_2) to phosphatidylinositol 3,4,5 triphosphate (PIP_3). PIP_3 activates AKT, which blocks apoptosis and induces protein synthesis and cell proliferation. PTEN is a phosphatase that antagonizes PI3K activity. In many human tumors, PTEN mutations lead to increased cell survival and cell proliferation.

Fig. 7.16 The Patched pathway. (a) In the absence of ligand (Sonic hedgehog), Patched, a seven-transmembrane protein, inhibits Smoothened activity and the transcription factor GLI is inhibited by SUFU. (b) Upon SHH binding, Patched is activated, thereby relieving its suppression on Smoothened. Activated Smoothened induces the translocation of GLI to the nucleus and transcription of GLI-responsive genes. (c) Mutations in Patched or in SUFU, a negative regulator of GLI, activate the pathway constitutively in the absence of SHH.

Fig. 7.17 The WNT pathway. (a) In the absence of its ligand, the seven-transmembrane receptor Frizzled is inactive. The kinase GSK-3β phosphorylates beta-catenin in complex with APC. β-Catenin is rapidly degraded. (b) When activated by binding of its ligand, Wnt, activated Frizzled activates Dishevelled, which inhibits GSK-3 β. This results in the dissociation of the APC complex, accumulation of beta-catenin and its translocation to the nucleus, where in complex with the transcription factor TCF it induces transcription of TCF-responsive genes. (c) Mutation of APC or β-catenin induces the constitutive translocation of β-catenin in the nucleus and transcription.

Fig. 7.18 The RAS signaling pathway. Ras is activated by growth factor receptor signaling. Activated RasGTP is inactivated to RasGDP by a GAP protein such as NF-1, a tumor suppressor protein mutated in neurofibromatosis. Activated RasGTP induces two pathways; the RAF/MAP kinase and the PI3K pathways. The RAF/MAP kinase pathway induces proliferation by activating the transcription of D-type cyclins and Mdm2. The PI3K pathway activates AKT to enforce cell survival. The phosphatase PTEN opposes the activity of PI3K.

Fig. 8.1 (a) The rediscovery of apoptosis as a generally important mechanism using *C. elegans*, the tiny, transparent nematode worm. (b) Identifying cell death genes. (c) Genetic pathway for programmed cell death.

Fig. 8.2 The two pathways of apoptosis: death receptor (extrinsic) and mitochondrial (intrinsic) pathways. Here, the death receptor pathway is triggered following binding of FAS ligand (FASL) to the FAS receptor (FAS). FASL binding induces clustering of FAS, which in turn recruits the adaptor protein FADD (FAS-associated death domain) to form a complex called the death-inducing signaling complex (DISC). DISC then recruits and activates the initiator procaspase-8. Subsequently, activated procaspase-8 triggers a caspase cascade, activating downstream executioner caspases, such as caspase-3 and caspase-7, which ultimately kill the cell. However, caspase-8 can also activate the proapoptotic protein, BID (by cleavage to become truncated tBid), which promotes release of cytochrome *c* from the mitochondria – the "intrinsic" pathway.

The mitochondrial pathway can be triggered by a variety of cellular stresses (e.g. DNA damage, hypoxia, depleted survival factors, or deregulated oncogenes). Once released into the cytosol, cytochrome *c* associates with APAF-1 to create the apoptosome, a complex that activates procaspase-9. In the presence of cytochrome *c* and the nucleotide dATP/ATP, procaspase-9 is autocatalytically activated and can now go on to activate downstream executioner caspases, such as caspase-3 and caspase-7. Other proteins that are released from the mitochondria include Smac/DIABLO (and probably Htra2/Omi), which bind to, and inhibit, IAPs thus preventing caspase-9 and caspase-3 inhibition.

Fig. 8.3 Functional domains of the APAF-1 protein. APAF-1 contains an N-terminal CARD by which it interacts with procaspase-9; a nucleotide binding domain (NBD); and a long carboxy-terminal extension containing 13 repeats of the WD40 motif. The WD40 motif is a conserved protein domain, approximately 40 residues long, that has a characteristic tryptophan-aspartate motif, and is thought to negatively regulate APAF-1.

Fig. 8.4 Formation of the apoptosome. In an individual APAF-1 molecule, two groups of WD40 repeats in the carboxy-terminal region are thought to keep the protein inactive until cytochrome *c* engages the repeats. Association with cytochrome *c* causes APAF-1 to convert from a "closed" conformation to a more "open" one, thus allowing the nucleotide dATP/ATP binding activity to be greatly facilitated. This binding triggers formation of the active seven-span symmetrical "wheel of death" – the apoptosome – via interaction among the N-terminal CARD of the individual APAF-1 molecules. The apoptosome subsequently recruits procaspase-9 into its central hub through CARD–CARD domain interaction – between procaspase-9 and APAF-1 molecules. An inactive procaspase-9 monomer on one spoke of the apoptosome is thought to recruit another monomer to create a dimer with a single active site. This active caspase activates downstream executioner caspases, such as caspase-3 and caspase-7.

Fig. 8.5 Initiator and executioner caspases. Caspases are highly selective **c**ysteine proteases that have a preference for cleaving proteins after aspartate residues (arrows). Initiator procaspases, such as procaspase-8 and procaspase-9, possess long prodomains that contain a protein interaction domain, such as the death-effector domain (DED) and CARD, respectively. These domains mediate the homophilic interactions (i.e. CARD–CARD and DED–DED) between procaspases and their adaptor proteins (e.g. APAF-1 with procaspase-9; FADD with procaspase-8) and are indispensible to the activation of initiator caspases. Executioner procaspases, such as procaspase-3, have short prodomains that seem to inhibit caspase activation. Procaspase-3 is activated by initiator caspases: it is cleaved at specific Asp residues to yield a short inhibitory prodomain and a large and a small subunit, termed homodimers p20 and p10, respectively. Subsequently, p20 and p10 homodimers interact in a heterodimer – the association of two heterodimers forms the proteolytic tetramer with the two adjacent p10 subunits surrounded by two large subunits.

Fig. 8.6 Proteins of the IAP family. XIAP, an IAP family member, contains three baculoviral IAP repeat (BIR) domains. The third BIR domain (BIR3) potently inhibits the activity of processed caspase-9, whereas the linker region between BIR1 and BIR2 specifically targets caspase-3 and caspase-7. On the other hand, Survivin contains a single BIR domain and does not inhibit caspase activity *in vitro*.

Fig. 8.7 The BCL-2 family. The BCL-2 family of proteins can be subdivided into three categories according to their structure and function: the antiapoptotic members promote cell survival, whereas members of the BAX family and 'BH3-only' categories are proapoptotic and thus promote apoptosis. The BCL-2 family members possess at least one of four conserved motifs known as BCL-2 homology domains (BH1–BH4). Most members have a carboxy-terminal hydrophobic domain (TM) that aids association with intracellular membranes. Pore formation is enabled by residues of BH1, BH2, and BH3. During apoptosis, the proapoptotic members are activated, and presumably undergo a conformational change leading to exposure of the BH3 domain – an interaction domain that is necessary for their killing action. In this way, BH3-only proteins promote apoptosis by directly binding to their antiapoptotic relatives.

Fig. 8.8 BH3-only proteins kept inactive in healthy cells. BIM and BMF are sequestered by binding to dynein light chains that are associated with the microtubules and actin cytoskeleton. BAD is kept inactive when phosphorylated by kinases, such as AKT and protein kinase A, through being bound by 14-3-3 scaffold proteins (proteins that provide a platform for the assembly of other proteins). BID is inactive until proteolytically cleaved, for example, by caspase 8 or granzyme B.

Fig. 8.9 Regulation of MOMP by the BCL-2 family. In healthy cells, mitochondrial membranes remain intact. Antiapoptotic members BCL-2 and its closest homologs, BCL-x_L and BCL-w, are potent inhibitors of apoptosis in response to many stress signals. They protect mitochondrial integrity and thus prevent the release of cytochrome *c*, as well as other proapoptotic molecules (DIABLO/Smac and Omi/Htra2). BCL-2 is an integral membrane protein in all cells, whereas BCL-x_L and BCL-w only become tightly associated with the membrane after a cytotoxic signal, possibly through conformational change.
During apoptosis, proapoptotic "BH3-only" proteins neutralize the activity of BCL-2. Then, proapoptotic members, BAX and BAK, form homo-oligomers within the mitochondrial membrane and induce MOMP allowing efflux of cytochrome *c* and the formation of the apoptosome. BAX is normally a cytosolic monomer but translocates to the mitochondrial membrane during apoptosis and changes conformation forming homo-oligomers within the outer mitochondrial membranes. BAK, however, is an oligomeric integral mitochondrial membrane protein but also changes conformation during apoptosis and might form larger aggregates. The release of DIABLO/Smac and Omi/Htra2 from the mitochondria permits them to bind to and inhibit IAPs – proteins that prevent caspase-9 and caspase-3 activation.

Fig. 8.10 p53 and apoptosis. The tumor-suppressor protein, p53, can promote apoptosis in a number of ways. There are several proapoptotic transcriptional targets of p53, such as BAX and BH-3 proteins, NOXA, and PUMA, that promote cytochrome *c* release from the mitochondria. p53 can also downregulate the transcription of genes that inhibit apoptosis, including the IAP-family member, Survivin. p53 protein has also been shown to antagonize the antiapoptotic proteins BCL-2 and BCL-x_L, at the mitochondrial outer membrane, by directly binding to them.

Fig. 8.11 Potential responses to anticancer therapy. Anticancer therapy causes DNA damage to tumor cells. Depending on cell type and the genetic mutations acquired, a given tumor cell may respond to such damage in one of the following ways: die by apoptosis, mitotic catastrophe, autophagy, or necrosis (nonapoptotic cell death). Elimination is of course the most favorable outcome. Alternatively, the cell may enter a state of "permanent" cell cycle arrest (cellular senescence). However, it remains debatable as to whether the senescent cell promotes growth of neighboring tumor cells by secreting growth factors, or whether it will eventually re-enter the cell cycle. The most unfavorable outcome is obviously resistance to therapy as the cell is unable to undergo apoptosis or cellular senescence.

Fig. 8.12 Pathways to resistance. Whether a tumor cell responds favorably (cell death or cellular senescence) or unfavorably (resistant) to anticancer therapy will depend on the genetic mutations acquired during tumor development. For example: (a) in the early stages of tumorigenesis, if the initiating activating oncogene is *c-MYC* (where apoptosis is the "in-built" fail-safe mechanism), an apoptosis defect such as *BCL-2* overexpression will now prevent cells from dying by apoptosis. Despite this, such tumor cells may respond favorably by entering a state of cellular senescence. Further mutations, however, may also prevent cellular senescence, for example, inactivating *p53* or *INK4A* mutations, leading to resistance. Another example (b) shows tumor cells that have acquired an initiating oncogenic *RAS*, where cellular senescence is the fail-safe mechanism. Inactivating mutations in *INK4A* tumor-suppressor gene prevent senescence in response to anticancer therapy. However, cells can still undergo apoptosis due to the presence of intact p53. Not surprisingly, the loss of p53 prevents tumor cells dying by apoptosis and the tumor is now resistant to anticancer therapy.

Fig. 9.1 The nonhomologous end joining (NHEJ) complex DNA-PK has been involved both in mediating telomere fusions and apoptosis triggered by critically short telomeres. In particular, these consequences of telomere dysfunction are rescued in DNA-PKcs and Ku86-deficient backgrounds. However, cell cycle arrest due to short telomeres is not rescued by the absence of DNA-PKcs and Ku86 proteins. Blasco *et al.* have suggested that p53 may be downstream of DNA-PK in signaling apoptosis due to short telomeres.

Fig. 9.2 Different activities modulate telomere length, such as the telomere-binding proteins TRF1 and TRF2. More recently, activities involved in DNA repair (Ku86 and DNA-PKcs), as well as in cell cycle regulation (p107 and p130) have been also shown to have a direct impact in regulating telomere length. Ku86 is a negative regulator of telomerase, while DNA-PKcs cooperates with telomerase in maintaining telomere length. Simultaneous absence of p107 and p130 results in a fast elongation of telomeres in the absence of changes in telomerase activity.

Fig. 9.3 Activation of RAS and MYC results in potential engagement of both replication and growth but also of apoptosis and possibly senescence. If either MYC or RAS levels are excessive (as might occur during onogenesis) or other proapoptotic signals are received then the balance may be tipped away from replication. RAS can promote senescence through either $p16^{INK4a}$ or ARF, which activate RB or p53 pathways respectively. Senescence may also result from telomere erosion or dysfunction which may activate DNA damage responses that in part are the same as those also activated by DNA DSBs. Functional telomeres must normally prevent DNA ends being recognized and processed as DSBs. MYC can inhibit growth arrest/senescence by inhibiting $p21^{CIP1}$ and inducing TERT (telomeres reverse transcriptase), which prevents erosion of telomeres and may activate replication by other means also. Although MYC may activate the apoptotic pathway (e.g. via ARF), RAS is able to suppress apoptosis by activating the PI3K pathway and, subsequently, AKT. It can readily be appreciated how oncogenic MYC and RAS may conspire in oncogenesis. The combination of RAS and MYC acting together provides a potential means of avoiding apoptotic and senescence mechanisms activated by either acting individually. Moreover, it can also be appreciated how inactivating mutations in RB or p53 (or their pathways involving $p19^{Arf}$, $p16^{INK4a}$, $p21^{CIP1}$, etc) may contribute to tumorigenesis by enabling the cancer cell to avoid either senescence or apoptosis or both.

Fig. 9.4 Meiotic telomeres. Fluorescence *in situ* hybridization (FISH) showing a meiocyte nucleus where chromosomes are stained in red with a synaptonemal complex protein, and telomeres are stained in yellow with a PNA-telomeric probe. (Please see the website for the color version of this figure.)

Fig. 9.5 Metaphase chromosomes. Fluorescence *in situ* hybridization (FISH) of a mouse metaphase nucleus showing the chromosomes in blue (DAPI staining), and the telomeres in yellow (staining with a PNA-telomeric probe). (Please see the website for the color version of this figure.)

Fig. 9.6 Mouse chromosomes. Spectral karyotyping (SKY) to visualize mouse chromosomes. (Please see the website for the color version of this figure.)

Fig. 9.7 Telomere-interacting proteins. A number of proteins are bound to telomeres and have been shown to be involved in regulating telomere length and telomere capping.

Fig. 9.8 Telomere heterochromatin. Model for the assembly of telomeric heterochromatin. The Suv39h histone methyltransferases trimethylate lysine 9 at histone 3 (H3K9), which in turn creates a binding site for the heterochromatin protein 1 (HP1). These modifications confer heterochromatic features to telomeric chromatin, which in turn may impede the access of telomerase or other telomere elongating activities to the telomere, thus regulating telomere length.

Fig. 10.1 Cellular responses to DNA damage. Following DNA damage the cell may undergo one of three fates, repair and survive, fail to repair and apoptosis, or replicative senescence. Rarely the cell with damaged DNA may still be able to replicate and thus pose a threat of cancer. The molecular processes activated by DNA damage are shown in the second figure.

Fig. 10.2 Potential causes of the "mutator" phenotype. Mutations in multiple pathways can result in the mutator phenotype in cancer cells. From Loeb, L.A., Loeb, K.R., and Anderson, J.P. (2003). Multiple mutations and cancer. *Proceedings of the National Academy of Sciences USA*, **100**(3): 776-81. Epub: January 27, 2003.

Fig. 10.3 Normal human SKY profile. Images courtesy of the NIH Human Genome Resource.

Fig. 10.4 DNA-damage-induced signaling. Signaling mechanisms are activated by DNA-damage sensors, ultimately leading to a variety of potential cellular responses discussed earlier.

Fig. 10.5 Signaling involved downstream of different types of DNA damage. To combat recurrent threats of genomic instability, numerous distinct enzyme systems sense DNA damage and coordinate its repair. Part of this coordination involves the activation of signal transduction cascades that target repair proteins, trigger DNA-damage-dependent cell cycle checkpoints and profoundly affect chromatin neighboring a DSB. Ultimately DNA damage results in cell cycle arrest and attempted repair. If repair is successful, the cell will survive and may reenter the cell cycle. There is obvious overlap between telomere maintenance and DNA damage response. Proteins directly involved in telomere maintenance and DNA damage response include Ku, DNA-PKcs, RAD51D, PARP-2, WRN and MRE11/ RAD50/NBS1 complex. The kinases ATM and ATR are key mediators of the DNA-damage response and activate downstream effectors such as γH2AX, CHK2, and CHK1. Ultimately the activity of these kinases results in activation of p53-p21, inhibition of Cdc25 and activation of the proapoptotic BH3-only protien BID leading to growth arrest or apoptosis. 14-3-3σ is a p53 target which may also help maintain G2 arrest. Other 14-3-3 family members may seuester CDC25. Recent studies suggest that P53CSV and/or 14-3-3σ may prevent apoptosis if DNA damage is repairable and activation of another protein the p53-regulated serine/threonine phosphatase, PPM1D, may allow cell cycle reentry if DNA damage is repaired. Cdc25B and PLK1 may also help restart the cell cycle.

Fig. 10.6 Base excision repair.

Fig. 10.7 Nucleotide excision repair.

Fig. 10.8 Mismatch repair.

Fig. 10.9 (a) Nonhomologous end-joining results in a change in the original DNA sequence – either deletions (shown here) or insertions. (b) Homologous recombination results in more accurate repair of DSBs but requires a more complex process.

Fig. 10.10 Oncogenic stress, induced by either oncogenic c-*MYC* or loss of RB. During tumorigenesis, the developing cancer cells experience "oncogenic stress", which evokes a counter-response to eliminate them. Although, some of the key signaling proteins involved in oncogenic stress have been known for some time, notably ARF, the nature of all the key signals distinguishing between regular cell cycles and cancer cycles are still not fully characterized. This figure summarizes what is currently known. Deregulated expression of c-*MYC* can activate various BH3-only proteins such as BIM and BID, which by sequestering antiapoptotic BCL-2 proteins cooperates with the ARF–p53–PUMA/Noxa/BAX pathway in inducing apoptosis. At least in part the activation of p53 and BID may be via DNA damage responses activated following c-*MYC* activation, suggesting that this may play a key role in activating these "failsafe" mechanisms not otherwise activated by c-*MYC* during normal cell cycles. BID may also contribute to arrest in S phase. Oncogenic c-*MYC* can result in excess production of ROS and DNA DSBs, which may be key triggers for MYC-induced apoptosis in this context. Downregulation of $p21^{CIP1}$ by c-MYC may also push cells from growth arrest towards apoptosis. DNA damage results in transcriptional induction of p53 target genes, including $p21^{CIP1}$ as well as the proapoptotic Bcl-2 family member p53 upregulated modulator of apoptosis (PUMA), Noxa, and BAX. $p21^{CIP1}$ and PUMA mediate cell cycle arrest and apoptosis, respectively. Importantly, $p21^{CIP1}$ may trigger cell cycle arrest at the expense of apoptosis, therefore suppression of $p21^{CIP1}$ by c-MYC may result in the predominance of apoptosis over cell cycle arrest as an inherent tumor suppressor activity. It is possible that other CKIs such as $p16^{INK4a}$, which can also growth arrest cells, might also prevent apoptosis by reducing MOMP. Loss of RB, which also deregulates cell cycle control, can also culminate in DSBs and apoptosis, probably via aberrant activation of E2F1. Dashed lines indicate the presence of intermediate proteins (known or unknown).

Fig. 11.1 Methylation can inhibit gene transcription. In the presence of CTCF the gene is "insulated" from methylation; in this case the gene is transcriptionally active as it will remain in the unmethylated state. Conversely, in heterochromatin CpG islands in the promoter region are methylated and these regions are transcriptionally inactive. Gene silencing following methylation is reinforced by deacetylation and interactions with repressors of transcription. In fact, DNMTs, which further mediate methylation, may be actively recruited to help maintain silencing. Methylation in turn enables the binding of a complex comprising the methylcytosine binding protein (MBP) and histone deacetylase (HDAC); some MBPs (MECP2 and MBD1 and 2) can also associate with transcriptional corepressors such as SIN3, which directly bind to HDAC, and contribute to gene silencing. HDAC promotes deacteylation of histones, which contributes to organization of nucleosomes, and also more generally repression of transcription. In order for gene expression to take place, the HDAC-SIN3 complex is displaced and a transcription activator complex (transcription factor, histone acetyl transferase-HAT- and coactivator protein) can associate with promoter elements; HAT acetylates the histones reversing effects of HDAC. In cancer many genes may be appropriately inhibited by methylation in the promoter region and this is a frequent cause of loss of tumor suppressor activity during tumorigenesis.

Fig. 11.2 Imprinting of the gene for IGF2. On chromosomes inherited from the female, a protein called CTCF binds to an insulator preventing interaction between the enhancer and the *IGF2* gene. *IGF2* is therefore not expressed from the maternally inherited chromosome. Because of imprinting, the insulator on the male-derived chromosome is methylated; this inactivates the insulator, by blocking the binding of the CTCF protein, and allows the enhancer to activate transcription of the *IGF2* gene. It is speculated that CTCF is displaced by another protein, BORIS. The methylation patterns (imprints) on the chromosome, inherited by the zygote after fertilization, are maintained in subsequent generations by maintenance methyl transferases. After Alberts *et al.*, 2002 *Molecular Biology of the Cell*.

Fig. 11.3 Regulation of gene expression by histone acetylation. Gene expression is also regulated by acetylation as well as by methylation. Acetylation of histones results in a more open configuration of chromatin allowing access of transcriptional activators to the gene to be transcribed. Conversely, deacetylation results in a more closed conformation of DNA, denying access to genes. In fact, displacement of HDAC may be one way in which RB phosphorylation enables access of E2F for transcription of S-phase genes in the cell cycle.

Fig. 11.4 The genetic and epigenetic route to colon cancer. Genetic instability (MIN, in particular) contributes to many colonic cancers. MIN may arise either through genetic or epigenetic mechanisms (or possibly a combination).

Fig. 11.5 RNAi. Cells can inhibit gene expression via small double-stranded RNA. The enzyme Dicer cuts short interfering RNAs (siRNA) from longer double-stranded RNAs deriving from (1) self-copying gene sequences, (2) viruses, or (3) regulatory RNA sequences called microRNAs. All these RNAs are cleaved by Dicer into short siRNA segments that can suppress gene expression. The short siRNA pieces unwind to form single-stranded RNAs, which combine with proteins to form a complex called RISC. The RISC captures native mRNA molecules with sequences complementary to the short siRNA sequence. If the pairing is perfect, the native mRNA is cleaved into untranslatable RNA fragments. If the pairing is not perfect then the RISC complex halts translation by preventing ribosome movement along the native mRNA.

Fig. 11.6 Structure of the proteasome. The core particle comprises two copies of each of 14 different proteins, assembled in groups of 7 forming a ring. There are four rings each stacked on the other. There are two identical regulatory particles at each end of the complex, each consisting of 14 different proteins distinct from those in the core particle, 6 of which are ATPases, and some with sites that recognize ubiquitin. Proteins destined for destruction have been conjugated to a molecule of ubiquitin by ligases onto lysine residues. Additional molecules of ubiquitin bind to the first forming a chain. The complex binds to ubiquitin-recognizing site(s) on the regulatory particle and the protein unfolds, which utilizes energy from ATP. The unfolded protein translocates into the central cavity of the core particle. Several active sites on the inner surface break specific peptide bonds to form peptides averaging about eight amino acids in length. These peptides are extruded and may either be further degraded in the cytosol or incorporated in a class I histocompatibility molecule to be presented to the immune system as a potential antigen.

Fig. 11.7 The role of PML in senescence pathways. Upregulation of promyelocytic leukemia protein (PML) results in relocation of CBP and p53 to the PML body. CBP acetylates p53 increasing activity and expression of p53-target genes promoting senescence. An intact PML body may not be a prerequisite for this. Conversely, SIRT1 expression may antagonize PML by promoting p53 deacetylation. PML is also associated with hypophosphorylated RB and reduced E2F transcriptional activity. As cells reach senescence, a change in chromatin structure, called SAHF (senescence-associated heterochromatin foci), silences the genes that promote growth. In addition to PML, two other proteins, HIRA and ASF1a, are also involved in formation of SAHF. After Langley *et al.*, *EMBO Journal* (2002) 21, 2383—2396.

Fig. 12.1 Tissue architecture. The key components of epithelial tissues are shown as (a) a three-dimensional representation of a pseudostratified epithelium and (b) a schematic cross-section of a simple epithelium. They contain epithelial cells, separated from the stroma by a basement membrane, stromal cells such as fibroblasts and adipocytes, capillaries, neurons, and cells of the immune system. Although the main cell type to become altered genetically, and therefore neoplastic, is the epithelial cell itself, cancer progression depends on influences from other cell types. Thus, cancer is a disease where homeostasis of a whole organ is disrupted. It involves not just the cancer cells themselves, but is characterized by disturbed stromal, endothelial, and immune cells, as well as altered ECM.

Fig. 12.2 Key adhesive interactions of epithelial cells. (a) Several types of cell–cell adhesion system bind adjacent cells together. Tight junctions form a ring around the apical surface of epithelia, and thereby ionically separate the extracellular spaces at the apical and basal surface. Adherens junctions connect cells via cadherins, and organize the microfilaments, particularly toward the apical side of polarized cells. They also sequester the transcription factor, β-catenin. Gap junctions provide ionic communication between adjacent cells. Desmosomes are large plaques that form strong intercellular bonds, again via cadherins. They are of key importance in maintaining tissue integrity under conditions of shear stress. The basal surface of epithelial cells interacts with glycoproteins within the specialized ECM, basement membrane, via both integrin receptors and transmembrane proteoglycan receptors (the latter are not shown). Integrins can either be located within multiprotein assemblies known as adhesion complexes, where they link to the actin cytoskeleton, or in very large adhesion plaques called hemidesmosomes, which link to the intermediate filament network, itself forming a bridge to the nuclear envelope. (b) In addition to binding cells to the ECM, integrin receptors regulate cell shape and polarity via the cytoskeleton, and they link to intracellular signaling pathways via structural adaptor proteins and signaling enzymes. Adherens junctions have a similar function in cytostructural regulation and signaling. Polarity: Most adhesive cells have an intracellular "direction," so that different components become spatially segregated to different sides of the cell. Epithelial cells have a basal surface, which contacts the basement membrane, and an apical surface at the opposite side of the cell. Plasma membrane lipids are different on apical and basal surfaces. Similarly, the cytoplasmic contents are different toward apical and basal poles and nuclei are usually located within the basal half.

Fig. 12.3 Three-dimensional culture models for cancer. Although many aspects of cell behavior, including survival and proliferation, are frequently studied in monolayer culture, these often do not reflect the responses of cells in three-dimensional arrays *in vivo*, and extreme caution has to be taken to interpret experimental data. Fortunately, culture models are now available that mimic the three-dimensional organization of tissues, and better models are therefore being developed to study carcinoma progression. ErbB2 is a member of the EGF receptor family and is frequently upregulated in breast cancer. However, an activated ErbB2 does not transform normal breast epithelial cells, as assessed by conventional methods such as growth in soft agar. In this example, (a) breast cells plated in three-dimensional basement membrane gels grow to form hollow acini surrounded by a collagen-IV-containing basement membrane, which resemble mammary alveoli *in vivo* (Fig. 12.14b,c). Under the same culture conditions (b) the activated ErbB2 now causes the cells to proliferate so that they fill the luminal spaces and it alters their properties so that they form multiacinar structures. These resemble some forms of early breast cancer, such as comedo ductal carcinoma *in situ* (Fig. 12.14d). From Muthuswamy, S.K., Li, D., Lelievre, S., Bissell, M.J., and Brugge, J.S. (2001). ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini. *Nature Cell Biology*, **3**: 785–92. Petersen, O.W., Ronnov-Jessen, L., Howlett, A.R., and Bissell, M.J. (1992). Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. *Proceedings of National Academy of Sciences, U S A.* **89**: 9064–8.

Fig. 12.4 Polarity segregates signaling ligands from their receptors and thereby controls proliferation. GF receptor signaling in simple epithelia can be regulated by polarized segregation of the ligand to a different plasma membrane compartment than the receptor. Airway epithelia express the receptor ErbB1–4 as well as one of its ligands, heregulin-α. (a) The ligand is expressed on the apical cell surface and is secreted into apical medium, while the receptor is only present basally, and cannot be activated unless heregulin-α is added ectopically to the basal cell surface or if tight junctions in intact monolayers are broken. Wounding the epithelium causes rapid heregulin-α-activated ErbB2 signaling. In this way, segregation of ligand and receptor provides an elegant mechanism for rapid GF receptor activation and tissue repair after epithelial injury. (b) Unfortunately, this has dire consequences if cellular polarity is disrupted in disease processes, because it can lead to unscheduled proliferation. For example, increased epithelial permeability in smoking-associated bronchitis can disrupt growth homeostasis and therefore contribute to tumor formation. From Vermeer, P.D., Einwalter, L.A., Moninger, T.O. *et al.* (2003). Segregation of receptor and ligand regulates activation of epithelial growth factor receptor. *Nature*, **422**: 322–6.

Fig. 12.5 Laminin signaling is required for oncogene-induced squamous cell carcinoma. (a) The main role of oncogenic Ras^{V12} in epithelial cells is to suppress apoptosis. However, it can also inhibit proliferation by increasing NF-κB activity. In epidermal keratinocytes NF-κB inhibits proliferation, even though it activates the cell cycle in other cell types. This brake on proliferation can be blocked by preventing NF-κB activity, which in this example is achieved by stable expression of IκB. Therefore the antiapoptotic effect of Ras^{V12} plus the proliferative response to reduced NF-κB activity cooperate in malignant tumor formation. If Ras^{V12} and IκB are expressed in human epidermal cells, which are then grafted onto mouse skin, the cells proliferate to form a tumor resembling squamous cell carcinoma. (b) However, this only occurs when the keratinocytes interact with laminin-5 in the epidermal basement membrane through β4 integrin receptors (left). If Ras^{V12} and IκB are expressed in epidermal cells from patients genetically deficient in either this form of laminin or the β4 integrin, then they are unable to drive tumor formation (right). These experiments show that there is an essential crosstalk between cell–ECM interactions and oncogenes in human tumor progression. In this case, it is likely to be because β4 integrin has a signaling role. β4 integrin is frequently upregulated in cancer, and may therefore have an important contribution in driving oncogenesis. Similar mechanisms may be important in other tumors as well. Further changes in adhesion molecules are necessary for other aspects of squamous cell carcinoma progression to full malignancy (see Fig. 12.20). From Dajee, M., Lazarov, M., Zhang, J.Y. *et al.* (2003). NF-kappaB blockade and oncogenic Ras trigger invasive human epidermal neoplasia. *Nature*, **421**: 639–43.

Fig. 12.6 The stroma contributes directly to epithelial malignancy. In this experiment, (a) the epithelium is removed from the mammary gland of a young 3-week-old mouse, leaving the mammary fat pads that consist of only the stromal components. (b) Eight weeks later, the mice are irradiated on one side only, and mammary epithelial cells are transplanted into both irradiated and control mammary fat pads. After a further 8 weeks, the cells in the nonirradiated glands proliferate and undergo developmental morphogenesis to form a normal-appearing ductal outgrowth. The cells in the irradiated side also form ductal outgrowths, but a large number of tumors also develop. The mammary cells injected are normal apart from harboring mutations in both p53 alleles, indicating that they are genomically unstable. Thus, ionizing radiation, which is a known carcinogen, causes changes in the stroma, which then facilitates the expression of neoplasia within the epithelial cell compartment. This type of communication between stroma and epithelium is often overlooked, but is of key importance in cancer progression. From Barcellos-Hoff, M.H. and Ravani S.A. (2000). Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. *Cancer Research*, **60**: 1254–60.

Fig. 12.7 Stromal–epithelial interactions in invasive behavior of tumor cells. Tumor cells can subvert the normal function of stromal cells so that they contribute toward malignant progression, even though they do not become altered genetically. Here, tumor cells secrete TGF-β, which recruits myofibroblasts to differentiate from fibroblasts. Myofibroblasts have two effects on carcinoma cells that encourage them to migrate through the stroma. First they secrete HGF, which causes the carcinoma cells to undergo a phenotypic transition so that they take on features of migratory mesenchymal cells. Second, myofibroblasts secrete tenascin into the stromal ECM: this is enhanced by TGF-β. As a consequence, the small GTPase Rac is activated within the tumor cells, while Rho is inhibited. These enzymes regulate the cytoskeleton so that the cells become more motile. Together, this epithelial–stromal–epithelial activation loop causes stationary carcinoma cells to become migratory. From De Wever, O., Nguyen, Q.D., Van Hoorde, L. *et al*. (2004). Tenascin-C and SF/HGF produced by myofibroblasts in vitro provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. *FASEB Journal*, **18**: 1016–8.

Fig. 12.8 Integrin heterodimers. There are at least 25 distinct integrin heterodimer pairs in total, made up from 18 α and 8 β subunits. Each is specific for a unique set of ligands. This diagram only shows a subset of the various ECM proteins bound by integrins. Some integrin heterodimers are promiscuous and bind several ligands, while several ECM proteins interact with cells through different integrins, depending on cell type and differentiation state. Coll, collagen I, III, IV; LM, laminins; FN, fibronectin; VN, vitronectin.

Fig. 12.9 Adhesion complexes are multiprotein assemblies at the cell–ECM interface. This fluorescence image of a cell shows the well-organized actin-based cytoskeleton (central panel) that defines the architecture of the cell, and adhesion complexes (left panel) where the cell interacts with ECM. (Please see our website for the color version of this figure.) In this case, the cell has been stained with an antibody specific for the phosphorylated, active form of focal adhesion kinase (otherwise known as $pp125^{FAK}$ or FAK). Note that the skeletal components of the cell begin at the adhesion complexes: that is, their "feet." The dotted lines indicate coincidence of focal adhesions with the tips of cytoskeletal cables. The schematic diagram of the adhesion complex includes transmembrane integrins, which link cells to the matrix. Integrin ligation activates proximal cytoskeletal adaptor proteins, such as talin and vinculin, signaling enzymes, for example, FAK, Src, and integrin-linked kinase (ILK), and signaling adaptors such as paxillin (PAX) and 130Cas that couple to further downstream pathways. These include the Ras-Erk and PI3K-PKB axes, PKC and PLCγ, and small GTPases including Rho, Rac, and Cdc42. A large number of other proteins (not shown) are also within adhesion complexes. Adhesion-regulated signaling enzymes have key roles in controlling many aspects of cell behavior, including migration, polarity, survival, proliferation, and differentiation.

Fig. 12.10 Integrins are essential for tumor formation. (a) The normal mammary ductal tree consists of epithelial cells that form tubular arrays. In nonpregnant animals, these ductal trees remain stable. (b) If the polyoma middle-T antigen (MT) is activated *in vivo*, multifocal tumors form within a few weeks. This is because the middle-T antigen activates numerous signal transduction pathways that are normally associated with GF receptor signaling, such as those involving c-Src, phosphatidyl inositol 3′-kinase, and the ras–Erk pathway. (c) If β1 integrin is deleted through conditional ablation of the gene, tumors do not form. This is most likely because the middle-T-driven signaling pathways need active integrins in order to function properly. From White, D.E., Kurpios, N.A., Zuo, D. *et al.* (2004). Targeted disruption of beta1-integrin in a transgenic mouse model of human breast cancer reveals an essential role in mammary tumor induction. *Cancer Cell*, **6**: 159–70.

Fig. 12.11 Adhesion complexes at the cell–cell interface. (a,b) These fluorescence images of epithelial cell sheets show the localization of adhesion molecules distributed to cell–cell contact sites. (a) Cells stained for E-cadherin (green), which is visible in a continuous ring at the lateral junction between cells. (b) Cells stained for plakoglobin (γ-catenin), which only localizes to desmosomes and therefore has a punctate appearance. (Please see the website for the color version of these images.) Schematic diagrams of (c) an adherens junction and (d) a desmosome. Adherens junctions connect to, and organize, the actin-based cytoskeleton via the adaptor proteins α- and β-catenin. p120 is an important regulatory molecule, while β-catenin has a dual function as it is also a transcription factor. Desmosomes are strong spot-welds between cells, holding them together. They link to the intermediate filament network and thereby couple the scaffolding between hemidesmosomes and adjacent cells.

Fig. 12.12 Steps in the transition between a benign and malignant tumor. (a) Cells become DNA-repair and apoptosis defective, and acquire oncogenic properties. Their proliferation is dysregulated and normal tissue architecture is partially disrupted as a consequence. (b) Some cells may lose cell–cell adhesivity, and have ragged borders. (c) Additional migratory properties lead to exit of tumor cells from the primary neoplastic lesion. Disruption of the basement membrane is an important marker for the transition from a benign tumor to malignancy. These steps are essential to initiate tumor spread.

Fig. 12.13 Mechanisms of tumor spreading. (a) Passage of malignant cells from the primary tissue, where a tumor forms, to a secondary site, via the circulation. (b) Steps in the migration of malignant breast cancer cells to the organs where they colonize and form metastases. 1 – normal breast lobules are attached to ducts; 2 – formation of benign tumor, that is, ductal carcinoma *in situ* (note that cells proliferate abnormally inside the ducts); 3 – early steps in malignancy, that is, invasion into stroma; 4 – high-grade malignant tumor, that is, transfer into lymph nodes; 5 – passage of aggressive tumor cells to blood system and then heart; 6 – proximal sites of distant spread to lung and bone; 7 – metastases to other organs such as liver. The appearance of breast cancer cells in the lymph nodes is a hallmark of malignancy. If the lymph nodes of breast cancer patients have enough tumor cells to be seen histologically by a pathologist, it is most likely that these individuals will have already acquired metastases, and their chances of still being alive within 5 years are not high.

Fig. 12.14 Tumor histology reveals aggressiveness. (a,b) Schematic diagram of a duct (a tube) and a lobule (a sphere) in mammary gland. Both are bilayered epithelia. Luminal epithelial cells (dark gray) are subtended by myoepithelial cells (light gray) and a basement membrane (thick black line). Some examples of the histology of breast tumors as they progress from benign to invasive carcinoma (please see our website for the color version of this figure). (c) Normal breast has three main compartments: lobules, ducts, and stroma. Here, lobules of epithelia (L) with central lumina are shown, together with part of a large duct (D). Note the discrete organization of lobular epithelial cells, and that the duct is lined with a simple layer of epithelium. Stroma (S) surrounds the epithelia. (d) Ductal carcinoma *in situ*. The simple bilayered epithelium of the duct has been replaced by large numbers of proliferating carcinoma cells (C). Note that the tumor has a discrete boundary with the stroma (S). (e) Advanced lobular carcinoma. This tumor arose from lobules. A large area of tissue is now taken up by proliferating cancer cells within the stroma (not visible). (f) Secondary tumor (T) metastasized to lymph node. The expansion of the tumor appears to be compressing the lymphocytes (Ly).

Fig. 12.15 Tumor dormancy and micrometastases. Some cells break off from the tumor prior to oncogene activation and settle in foreign sites. Although these cells might have altered adhesion mechanisms and are able to survive in an inappropriate microenvironment, they do not necessarily have a deregulated cell cycle. Thus, they have not acquired all the hallmarks of a malignant phenotype, and may remain dormant for many years. Subsequently, these cells evolve at secondary sites into metastatic lesions. An important implication of this is that an effective way to treat cancer may be to target altered proteins that occur within the metastatic lesion, rather than those that drive proliferation in the primary tumor.

Fig. 12.16 The loss of E-cadherin is rate-limiting for tumor progression. In an experimental model of cancer progression, tumors form in the insulin-secreting islets of Langerhans in the endocrine pancreas. Transgenic mice that express the transforming SV40 T and t antigens under the control of the insulin promoter (Rip1Tag2 mice), develop cancer in a characteristic fashion that progresses through adenoma to carcinoma. The T antigens are expressed continuously from embryonic day 8.5, and by 7 weeks after birth, adenomas form. A few weeks after this, the tumors become angiogenic, and by the 11th week they become invasive. Tumorigenesis occurs because T antigen both promotes proliferation, by inactivating the retinoblastoma protein, and suppresses the proapoptotic activity of p53. E-cadherin is expressed by the normal pancreatic epithelial cells and in adenomas, but is completely lost once the tumor has progressed to the carcinoma stage. However, if the Rip1Tag2 mice are crossed with those expressing E-cadherin under the control of the same promoter (Rip1E-cad mice), the incidence of carcinoma formation is dramatically reduced. Some carcinomas do form, but these have still all lost E-cadherin expression. The experiments provide direct evidence that E-cadherin loss is required for the transition from benign adenoma to malignant carcinoma. From Perl, A.K., Wilgenbus, P., Dahl, U., Semb, H., and Christofori. G. (1998). A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature*, **392**: 190–3.

Fig. 12.17 EMTs. (a) Epithelia are normally stable, forming interactions with each other and with the ECM. These cells are polarized, and not particularly motile. (b) During EMT, signals provided by stromal cells cause epithelial cells to lose contact with one another and to alter their interactions with ECM. (c) The cells acquire a motile phenotype and take on many characteristics of fibroblasts (i.e. mesenchymal cells), such as their morphology, and expression of fibroblast markers including intermediate filaments that are characteristic of those cells. Epithelial–mesenchymal transition is a highly conserved process that is required during embryonic development in order that mesenchymal cells can be formed from the primitive epithelia of early blastocysts. This is necessary for the formation of a three-layered embryo during gastrulation. However, if the EMT program is reactivated in normal epithelial cells of adult tissues, it can contribute to malignant progression.

Fig. 12.18 The key role of integrins in cell migration during metastasis. Melanoma cells are highly metastatic and can cause rapid patient mortality. In order for the cells to migrate and form metastases, they require integrin-mediated adhesion. ECM proteins contain specific amino acid recognition motifs to be bound by integrins, and one of these contains the sequence arginine-glycine-aspartic acid (RGD). This recognition sequence is contained within ECM molecules that promote migration such as fibronectin and vitronectin, which are bound by $\alpha 5$ and αv integrins. In this example, melanoma cells cause experimental metastasis when injected into the tail veins of mice, but injecting an inhibitory RGD peptide at the same time can inhibit metastasis. This not only shows that integrin-mediated adhesion is necessary for metastasis to occur, it also indicates that strategies to block cell–matrix adhesion might be useful for cancer therapy. From Humphries, M.J., Olden, K., and Yamada, K.M. (1986). A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. *Science*, **233**: 467–70.

Fig. 12.19 MMPs control the proliferative response of tumor cells constrained within a three-dimensional ECM. (a) Cells that are attached to a substratum, but are forced into a rounded configuration, are unable to respond to GFs and do not proliferate. Rounded cells are, instead, prone to undergo apoptosis. This indicates that cellular geometry is critical in determining whether cells can proliferate or not. (b) When tumor cells are plated in two-dimensions on a culture dish coated with collagen (left panel) the cells are stretched out and they can proliferate well, but inside a three-dimensional collagen gel (right panel) the cells are rounded and their proliferation is severely compromised. This is because of low cyclin D3-kinase activity. However, if the cells express a surface-bound MMP, MT1-MMP, they clip the collagen fibers that enclose them, and after doing so they reorganize their cytoskeleton, stretch out, and now respond to proliferative signals. Thus, MT1-MMP allows cells that would otherwise be entrapped within a stromal or dermal matrix to proliferate, thereby providing a growth advantage. Indeed, the tumor cells expressing MT1-MMP grow much more quickly *in vivo* than those without it. This highlights the dramatic differences in growth potential of cells growing on a two-dimensional surface and the same cells embedded within a three-dimensional gel of the same matrix. As carcinoma cells exit a primary tumor and enter the stroma, they need to adapt to foreign signals and survive in the new ECM environment. They also need to proliferate, but can be prevented from doing so by the physical constraints of the stroma. The expression of MMPs provides a mechanism for carcinoma cells to proliferate at foreign sites that are frequently rich in dense networks of collagen or fibrin, and this is necessary for them to progress to full metastasis. MMP inhibitors may therefore have a dramatic therapeutic potential for cells growing in a three-dimensional environment similar to that *in vivo*, even if they have little effect on monolayer-cultured cells. From Chen, C.S., Mrksich, M., Huang, S., Whitesides, G.M., and Ingber, D.E. (1997). Geometric control of cell life and death. *Science*, **276**: 1425–1428. Hotary, K.B., Allen, E.D., Brooks, P.C., Datta, N.S., Long, M.W., and S.J. Weiss. (2003). Membrane type I matrix metalloproteinase usurps tumor growth control imposed by the three-dimensional extracellular matrix. *Cell*, **114**: 33–45.

Fig. 12.20 An integrin switch can suppress anoikis and participate in the progression of skin cancer. (a) Keratinocytes normally express $\alpha 2\beta 1$, $\alpha 3\beta 1$, and $\alpha 6\beta 4$ integrins, and have low levels of the αv integrins, usually $\alpha v\beta 5$. $\beta 1$ integrins are essential for stem cell maintenance in the basal layer, but as keratinocytes differentiate, the cells migrate away from the basement membrane into the suprabasal stratifying layers. Here, they are able to survive in the absence of integrin ligation and undergo terminal differentiation. Keratinocytes are uniquely different to other epithelia in this respect. (b) Under conditions where integrins are not ligated, cancerous keratinocytes expressing $\alpha v\beta 5$ integrin undergo apoptosis. The mechanism for this appears to be through a suppression of PKB, or Akt via the integrin $\beta 5$ subunit, though the details of how the integrin links to the PKB pathway are not yet clear. It probably acts as a fail-safe device to delete keratinocytes that are unable to differentiate properly. During the subsequent transition to squamous cell carcinoma, there is an integrin switch that results in elevated $\alpha v\beta 6$ integrin and a corresponding decrease in the $\alpha v\beta 5$ heterodimer. This leads to reactivation of PKB and suppression of anoikis. This is a unique mechanism to allow growth of tumor cells as they progress through the stroma. $\alpha v\beta 6$ integrin also contributes to invasion of squamous cell carinoma, because it promotes increased expression of MMPs. Some integrins may therefore have multiple roles in cancer progression, and in the case of squamous epithelium, altered integrin profiles can both protect cells from apoptosis and drive invasion. From Janes, S.M. and Watt, F.M. (2004). Switch from alphavbeta5 to alphavbeta6 integrin expression protects squamous cell carcinomas from anoikis. *Journal of Cell Biology*, **166**: 419–31. Thomas, G.J., Lewis, M.P., Hart, I.R., Marshall, J.F. and Speight, P.M. (2001). AlphaVbeta6 integrin promotes invasion of squamous carcinoma cells through up-regulation of matrix metalloproteinase-9. *International Journal of Cancer*, **92**: 641–50.

Fig. 12.21 The proapoptotic protein, Bax, regulates anoikis in epithelia. (a) In normal adherent epithelial cells, Bax is located within the cytosol (revealed by transfected YFP-Bax). The mitochondria are discrete. (b) When the same cells are detached from the ECM so that integrins are unligated and therefore unable to send survival signals, all the cytosolic Bax translocates rapidly to mitochondria. This can be seen by codistribution of YFP-Bax with the mitochondrial marker HSP70 (red). [Please see our website for our color versions of (a) and (b).] (c) This redistribution of Bax is schematized, showing relocalization of Bax to mitochondria. In an experimental situation, this process occurs homogeneously throughout a cell population. Cytochrome *c* remains within mitochondria for a long time after Bax translocation and eventually is released stochastically into the cytosol. Cells commit to apoptosis immediately after cytochrome *c* release. (d) Gene transfer experiments with plasmids expressing a dominant-negative version of FAK followed by rescue with constitutively active forms of Src, PI3K identify an integrin-triggered pathway that regulates the subcellular distribution of Bax. (e) Different cell types activate different intracellular signaling pathways downstream of integrins. FAK-mediated pathways suppress Bax in epithelia and p53 in fibroblasts, while an integrin pathway has effects on both Fas and c-FLIP in endothelial cells. From Gilmore, A.P., Metcalfe, A.D., Romer, L.H., and Streuli, C.H. (2000). Integrin-mediated survival signals regulate the apoptotic function of Bax through its conformation and subcellular localization. *Journal of Cell Biology*, **149**: 431–46. Ilic, D., Almeida, E.A., Schlaepfer, D.D., Dazin, P., Aizawa, S., and Damsky, C.H. (1998). Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis. *Journal of Cell Biology*, **143**: 547–60. Aoudjit, F. and Vuori, K. (2001). Matrix attachment regulates Fas-induced apoptosis in endothelial cells: a role for c-flip and implications for anoikis. *Journal of Cell Biology*, **152**: 633–43.

Fig. 13.1 Innate antitumor immunity. Development of tumor cells results in a proinflammatory milieu and activation of NK, NKT, and IEL (intraepithelial) cells. Release of cytokines and chemokines leads to recruitment of macrophages and additional innate effectors that provide an initial line of defense, and DCs that initiate the adaptive immune response.

Fig. 13.2 Adaptive anti-tumor immunity. DCs take up tumor antigen, and process and present antigen to CD4 and CD8 T cells in draining lymph nodes. Presentation of tumor antigen leads to CD4 and CD8 T-cell activation (cf. Fig. 13.3 for details). Activated CD4 and CD8 T cells traffic to tumor sites where they mediate antigen-specific effector response through the release of cytotoxic granules, Fas – FasL interaction, and recruitment of secondary effectors (cf. Fig. 13.4 for details).

Fig. 13.3 Antigen presentation. In the Class I pathway (① – ⑤), cytosolic proteins are processed by proteasomes ② into peptide fragments, transported through the endoplasmic reticulum (ER, ③), complexed with Class I MHC ④ and β2-microglobulin, and brought to the surface ⑤ where they are presented to CD8 T cells. In the Class II pathway, extracellular protein antigens are endocytosed ❶, degraded into peptide fragments ❷, combined with MHC ❸, and presented to the surface ❹ where they are presented to CD4 T cells ❺.

Fig. 13.4 Effector mechanisms of T cells. Activated CD8 T cells deliver a "death" signal to tumor cells through FasL – Fas interaction ①. CD8 T cells may also kill tumor cells directly through perforin and granzymes released upon engagement of the TCR②. Perforin exocytosed in CTL granules forms pores in the tumor cell membrane. Granzymes enter tumor cells through pores and induce tumor cell death③. CD4 T cells can mediate tumor death through Fas interaction. Activated CD4 T cells may also mediate cytotoxicity indirectly through the release of interferon-γ and IL-5 to recruit tumoricidal macrophages (mϕ) and eosinophils (Eos) ④.

Fig. 13.5 Identification of T-cell-defined tumor antigens. CD8+ T cells are stimulated *in vitro* with autologous tumor cells to generate tumor-specific CD8 T-cell clones ①. To identify the tumor antigen recognized by these T-cell clones, a cDNA library is first prepared from the tumor cell line ② and used to transfect HLA-matched nontumor target cells (in this case, COS cells) ③. These cells are screened using the tumor-specific T-cell clone ④ and those recognized by the T cell are isolated and their cDNA extracted, sequenced, and compared with a genetic database ⑤ to identify the antigen targeted by the tumor-specific T cell.

Fig. 13.6 Mechanisms of antibody-mediated killing. NK cells ❶ and macrophages ❷ docking through their Fc receptors to the Fc segment of antibodies bound to tumor cell surface antigen mediate cell lysis, a process known as ADCC. Alternatively, complement activation through the Fc portion of antibodies leads to a cascade of events resulting in a cell lytic complex that inserts itself into the cell membrane leading to tumor cell death ❸. Antibodies bound to radionuclides or toxins mediate cell death following internalization ❹. Blockade of growth factor receptors and/or activation of downstream signals lead to apoptosis and cell death ❺.

Fig. 14.1 Blood vessels are comprised of endothelial and mural cells. Double label immunofluoresence reveals that capillaries are formed by an inner layer of PECAM-positive endothelial cells and an outer layer of smooth muscle actin-positive pericytes.

Fig. 14.2 The classes of secreted or cell-surface-associated molecules that cooperate to stimulate vascular growth and differentiation. The life cycle of endothelium may begin with the assembly of angioblasts into a primitive vascular plexus (vasculogenesis), or with the reactivation of quiescent vessel endothelium. Through the process of sprouting growth (angiogenesis), the vessel network begins to expand. New vessels acquire mural cells and adopt an arterial or venous identity; concomitantly, the vascular network continues to branch and remodel into a hierarchical vascular tree. Lastly, vessels specialize according to local physiological needs, for example to form the blood-brain barrier or the fenestrated sieve plates found in the kidney glomerulus. With the notable exception of blood-brain barrier function, most of the processes illustrated are stimulated by VEGF; several other factors act upstream of VEGF or cooperate with VEGF to affect specific aspects of vessel growth and specialization.

Fig. 14.3 Angiogenic sprouting of blood vessels in the developing brain. Isolectin B4-positive blood vessels extend filopodia-studded sprouts to initiate new branch formation during brain vascularization in the mid-gestation mouse embryo.

Fig. 14.4 An experimental proof of principle – the importance of tumor angiogenesis for malignant growth. In 1972, Judah Folkman's group performed seminal experiments to prove the importance of neoangiogenesis for malignant tumor growth in the eye (Gimbrone *et al.*, 1972): When small tumor fragments were implanted under the cornea, malignant tumors formed rapidly if the transplantation site was located adjacent to highly vascularized iris tissue. In contrast, when such tumor fragments were placed under the cornea, but within the avascular anterior chamber, tumor cells continued to proliferate for a little while, but these tumors switched to a dormant state once they reached 1 mm in diameter. The modification of this original transplantation approach led to the development of the first widely used model system for the identification of tumor-derived angiogenic factors, the rabbit cornea pocket assay.

Fig. 14.5 Schematic illustration of the human VEGF-A isoforms. The VEGF isoforms are generated by alternative splicing from a single copy gene, termed *VEGF-A*. The isoforms differ by the absence or presence of domains that confer binding to heparin sulfate proteoglycans and neuropilin receptors and are encoded by exons 6 (6a or 6b) and 7. The VEGF183 isoform contains a deletion within exon 6a. The VEGF121 isoform does not contain any exon 6 or exon 7 domain. The murine orthologs of VEGF189, VEGF165, VEGF145 and VEGF121 have been described; they are referred to as VEGF188, VEGF164, VEGF144 and VEGF120, because they are shorter by one amino acid.

Fig. 15.1 Photograph of carcinoma in sigmoid colon seen through colonoscope.

Fig. 15.2 CT scanner (top) and MRI scanner. Note much longer imaging tube in which patient lies in the MRI scanner.

Fig. 15.3 CT image of thorax showing heart in center with surrounding lung in which circular metastases are clearly seen.

Fig. 15.4 MRI image of brain. Note the high anatomical detail demonstrated. There is a large tumor in the pituitary region.

Fig. 15.5 Photograph of carcinoma of bronchus as visualized down bronchoscope. The site of the tumor is illustrated on the diagram to the right.

Fig. 15.6 Radioisotope bone scan. Note the increased uptake of radioactive tracer producing "hotspots" in the scapulae and limbs.

Fig. 16.1 Concept figure for the chapter.

Fig. 16.2 Survival curves for cells with different α/β ratios subjected to irradiation. The more steeply curving survival curve has a lower α/β ratio when fitted to the linear quadratic equation. Note log scale. SF = surviving fraction following irradiation.

Fig. 16.3 The radiotherapy planning process. The most common staging system used is the tumor node metastasis (TNM) system.

Fig. 16.4 Schematic representation of "volumes" in radiotherapy. The treatment volume includes tumor volume, potential areas of local microscopic disease around tumor, and margin of surrounding normal tissue and a margin added on account of physical considerations. In the figure, A shows gross tumor volume, B shows clinical target volume, C shows internal margin (which cannot be altered significantly), D shows setup margin (a physical concept with some scope for alteration), and E shows planning target volume.

Fig. 16.5 Mechanisms of action of the antimetabolites methotrexate and 5-fluorouracil. These agents both interfere with the folic acid (folate) cycle, which particularly compromises DNA synthesis in replicating cells.

Fig. 16.6 Simplification of the EGFR signaling pathway showing the site of action of the monoclonal antibodies and the small molecule tyrosine kinase inhibitors.

Fig. 17.1 Palliative care's place in the course of illness (National Consensus guidelines).

Fig. 17.2 WHO analgesic ladder.

Fig. 18.1 Information flow in the cell – from genome to proteome to cellular networks. On each level, variation has an effect on cell function and abnormal variation can underlie cancer.

Fig. 18.2 HapMap-Consortium (2003). The International HapMap Project. *Nature*. **426**: 789 – 96. Reproduced by permission of *Nature.*

Fig. 18.3 A section of a 2D-gel from smooth muscle protein extract. Y-axis is separated on molecular weight. X-axis is separated on isoelectric point.

Fig. 18.4 Gamma-scintigraphic images of normal and tumor bearing rats. *In vivo* images were obtained after ^{125}I-APP (^{125}I-aminopeptidase-P) monoclonal antibodies were injected intravenously. A difference in lung shape indicates tumor growth. From Oh, P., Li, Y., Yu, J., *et al.* (2004). Subtractive proteomic mapping of the endothelial surface in lung and solid tumors for tissue-specific therapy. *Nature*, **429**, 629 – 35. Reproduced by permission of *Nature*.

Fig. 18.5 Continues activiation profiles of different catogories of EGFR signaling effectors. (a) Key proteins involved in receptor internalization and endosomal trafficking. (b) Proteins from the Ras-MAP kinase pathways. (c) Proteins involved in actin remodeling. (d) Novel proteins found to be activated by the EGFR signaling pathway. From Blagoev, B., Ong, S.E., Kratchmarova, I. and Mann, M. (2004). Temporal analysis of phosphotyrosine-dependent signaling networks by quantitative proteomics. *Nature Biotechnology*, **22**, 1139 – 45. Reproduced by permission of *Nature*.

Fig. 18.6 Schematic overview of TAP tag procedure. A protein of interest is expressed in the cell with a TAP tag. Cultured cells are lysed and the complex is purified with the first affinity column (IgG-beads). A specific protease, TEV-protease, then cleaves the complex off the affinity column. The complex is eluted from the column onto the second affinity column and further cleaned up. Finally, the cleaned up complex is eluted off the calmodulin beads by EGTA and can be analyzed by MS. All interacting proteins can be identified. From Puig, O., Caspary, F., Rigaut, G., Rutz, B., Bouveret, E., Bragado-Nilsson, E., Wilm, M. and Seraphin, B. (2001). The tandem affinity purification (TAP) method: a general procedure of protein complex purification. Reprinted from *Methods*. **24**, 218–29. Copyright (2001), with permission from Elsevier.

Fig. 18.7 Connectivity map of the yeast proteome established through TAP-tagging approaches. More than 80% of the proteome is highly connected. Links were established between complexes sharing at least one protein. Individual complexes are color coded. Red is cell cycle; dark green is signaling; dark blue is transcription, chromatin structure and DNA maintenance; pink is protein and RNA transport; orange is RNA metabolism; light green is protein synthesis and turnover; brown is cell polarity and structure; violet is intermediate and energy metabolism; light blue is membrane biogenesis and traffic. (Please see our website for the color version of this figure.) From Gavin, A.C., Bosche, M., Krause, R. *et al.* (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature*, **415**, 141–7. Reproduced by permission of Nature.