Growth and the Cell Cycle in Cancer

Michael Lea

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Growth and the Cell Cycle in Cancer - Lecture Outline

1. Measurement of tumor growth
2. Growth fraction and cell doubling time
3. Cell cycle control
4. Checkpoint defects in cancer cells
5. Apoptosis
6. Differentiation
7. Telomerase
8. Terminal deoxynucleotidyl transferase
9. STATS
10. Growth factors.

Measurement of tumor growth

In experimental solid tumors growth is most easily measured if the tumors are transplanted subcutaneously. Size can then be measured with calipers in two dimensions. Calculation of the volume is complicated by the fact that tumors can have an irregular shape. Tumors can be weighed at the time of sacrifice but this only gives a single time point. Early growth may be exponential but then becomes linear as the tumor increases in size. Factors in the slowing growth can be

1. Decrease in the growth fraction
2. Increase in cell loss particularly if there is central necrosis
3. Nutritional depletion due to outgrowing the blood supply
4. Lengthening of the cell cycle time.

Measurement of tumor growth

Tumor growth in experimental animals can be monitored by

1.18-fluoro-2 deoxyglucose positron emission tomography (FDG PET)

2.Magnetic resonance imaging (MRI)

3.Bioluminescence imaging

4.Fluorescence imaging

FDG PET and bioluminescence imaging are able to detect smaller tumors.

The main disadvantage of the optical methods 3 and 4 is the requirement for tumor cells to express a reporter gene.

Table 3-1 Growth Parameters of Human Neoplasms and Normal Tissues

Cell Type	Labeling Index (%)	Estimated Cell Doubling Time (days)
Normal bone marrow myeloblasts	32-75	0.7-1.1
Acute myeloid leukemia	8-25	0.5-8.0
Normal B-cell lymphocytes	0 -1	14-21+
High-grade lymphoma	19-29	2-3
Normal intestinal crypts	12-18	1-2
Colon adenocarcinoma	3-35	1.6-5.0
Normal epithelium/pharynx	2-3	—
Squamous cell carcinoma of the nasopharynx	5-16	2-4
Normal epithelium/bronchus	—	9-10
Epidermoid carcinoma of the lung	5-8	8-10
Normal epithelium/cervix	4-8	—
Squamous cell carcinoma of the cervix	13-40	—
Ovarian carcinoma	3-20	5-6
Benign mole of skin	0.3	—
Malignant melanoma of skin	12.8	—

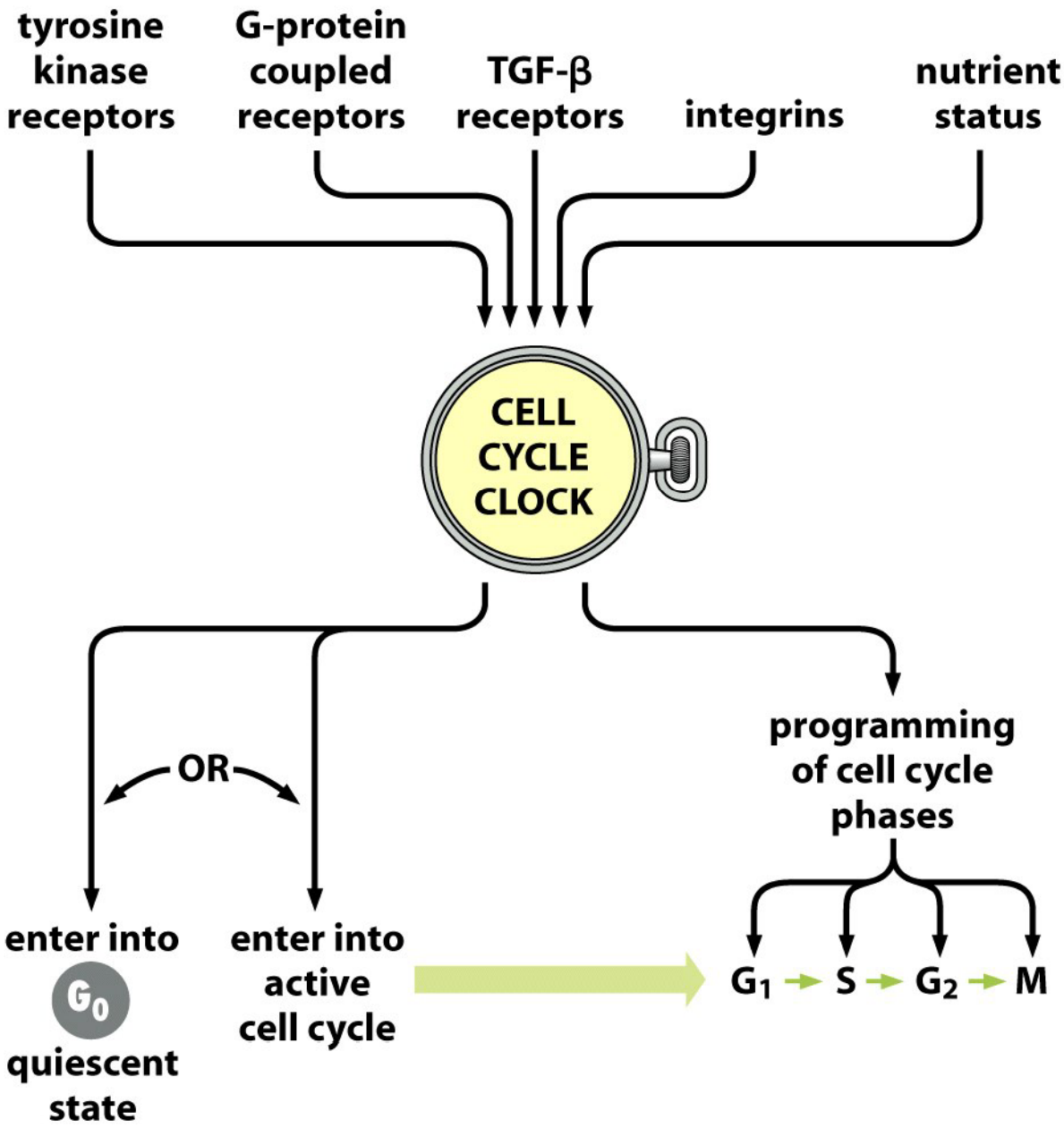


Figure 8-1 The Biology of Cancer (© Garland Science 2007)

Checkpoints in the cell cycle

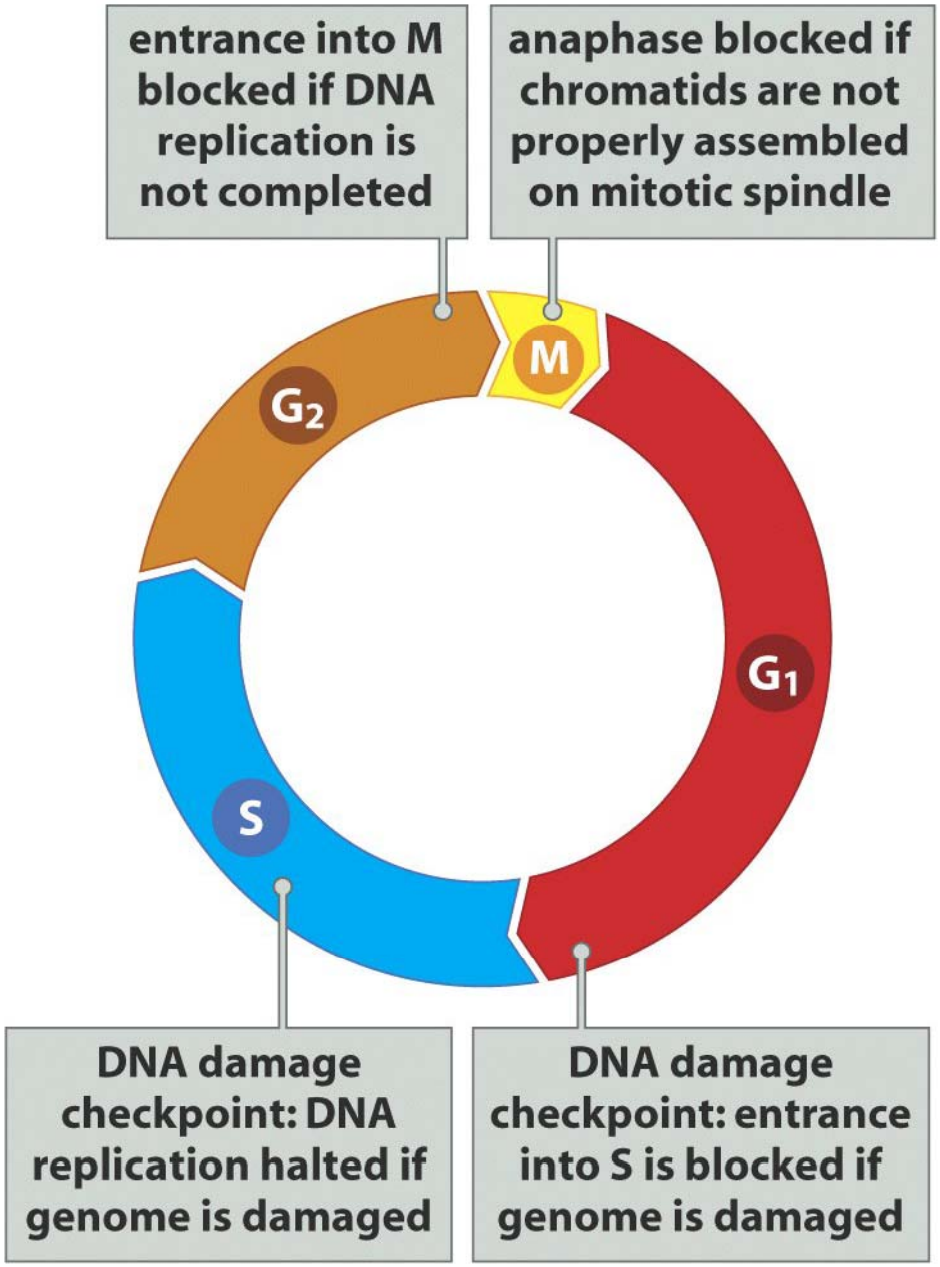


Figure 8-4 The Biology of Cancer (© Garland Science 2007)

Responsiveness to extracellular signals during the cell cycle ends in late G1 at the restriction point (R)

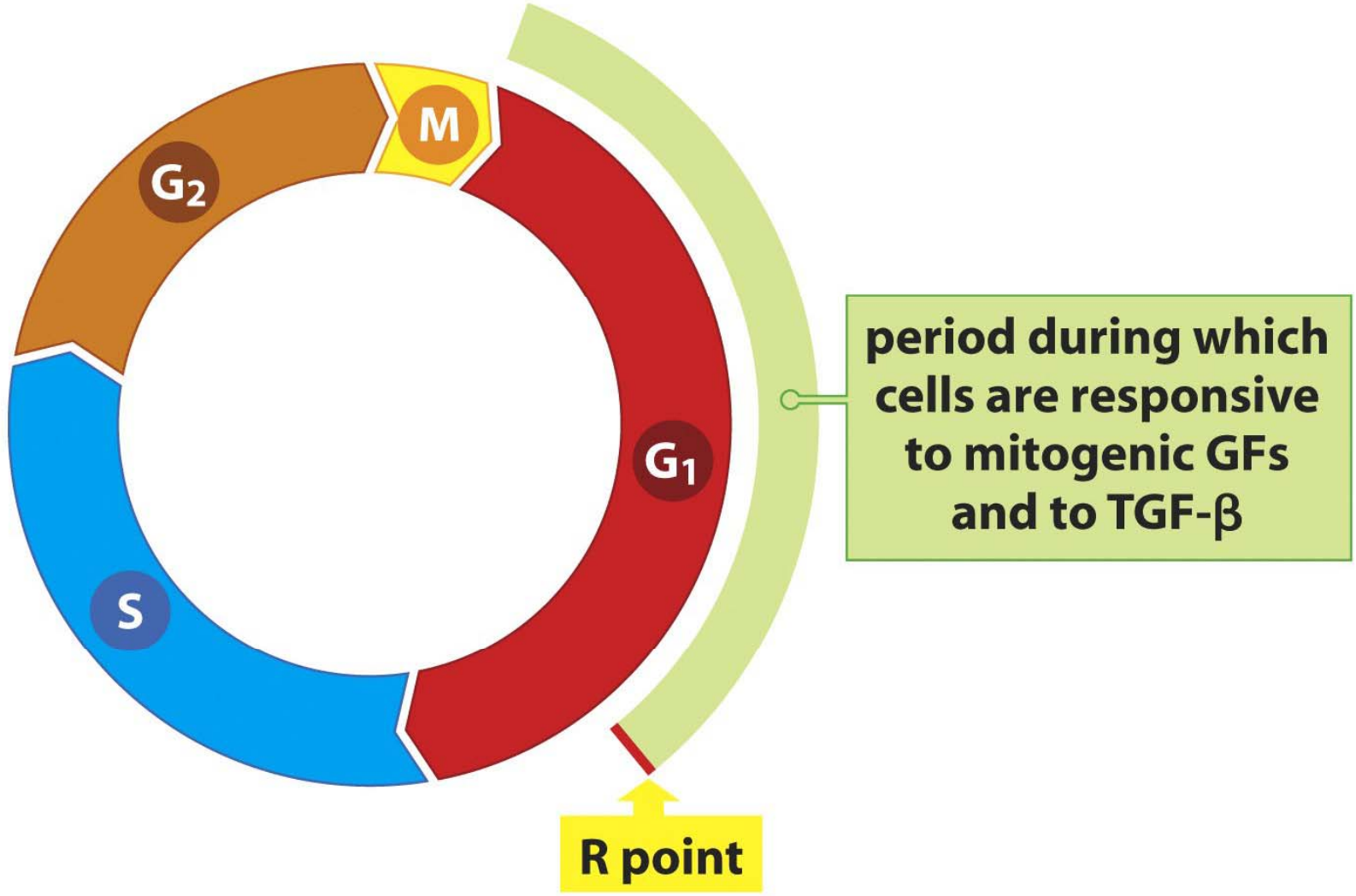
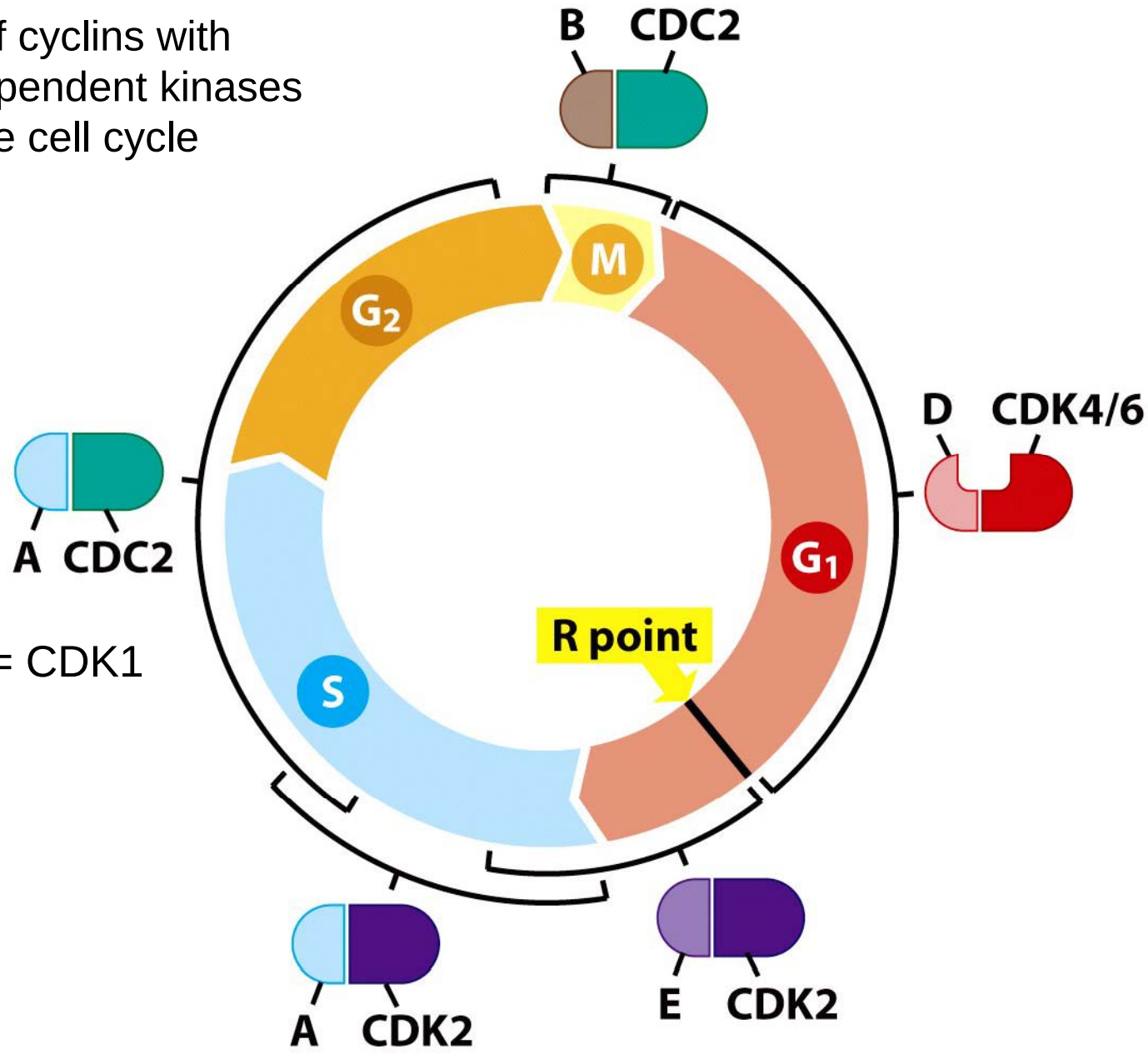


Figure 8-6 The Biology of Cancer (© Garland Science 2007)

Pairing of cyclins with cyclin-dependent kinases during the cell cycle



CDC2 = CDK1

Figure 8-8 The Biology of Cancer (© Garland Science 2007)

Checkpoint defects in cancer cells

Passage through the cell cycle requires the action of cyclin-dependent kinases and the changing levels of cyclins regulates the activity of these enzymes. In humans the following phase specific regulators are important:

Phase	Cdk	Cyclin
G1 (Go/S)	Cdk4/6	D
G1/S	Cdk2	E
S and G2	Cdk2	A
M	Cdk1	B

The over-expression of cyclin D1 has been detected in many human tumors owing to gene amplification or translocation of the gene to a different regulatory environment.

Loss or down-regulation of cyclin-dependent kinase inhibitors will favor cell cycle progression. One group including p21, p27 and p57 inhibit multiple Cdks while another group including p16 inhibits cyclin D/Cdk4 or Cdk6 .

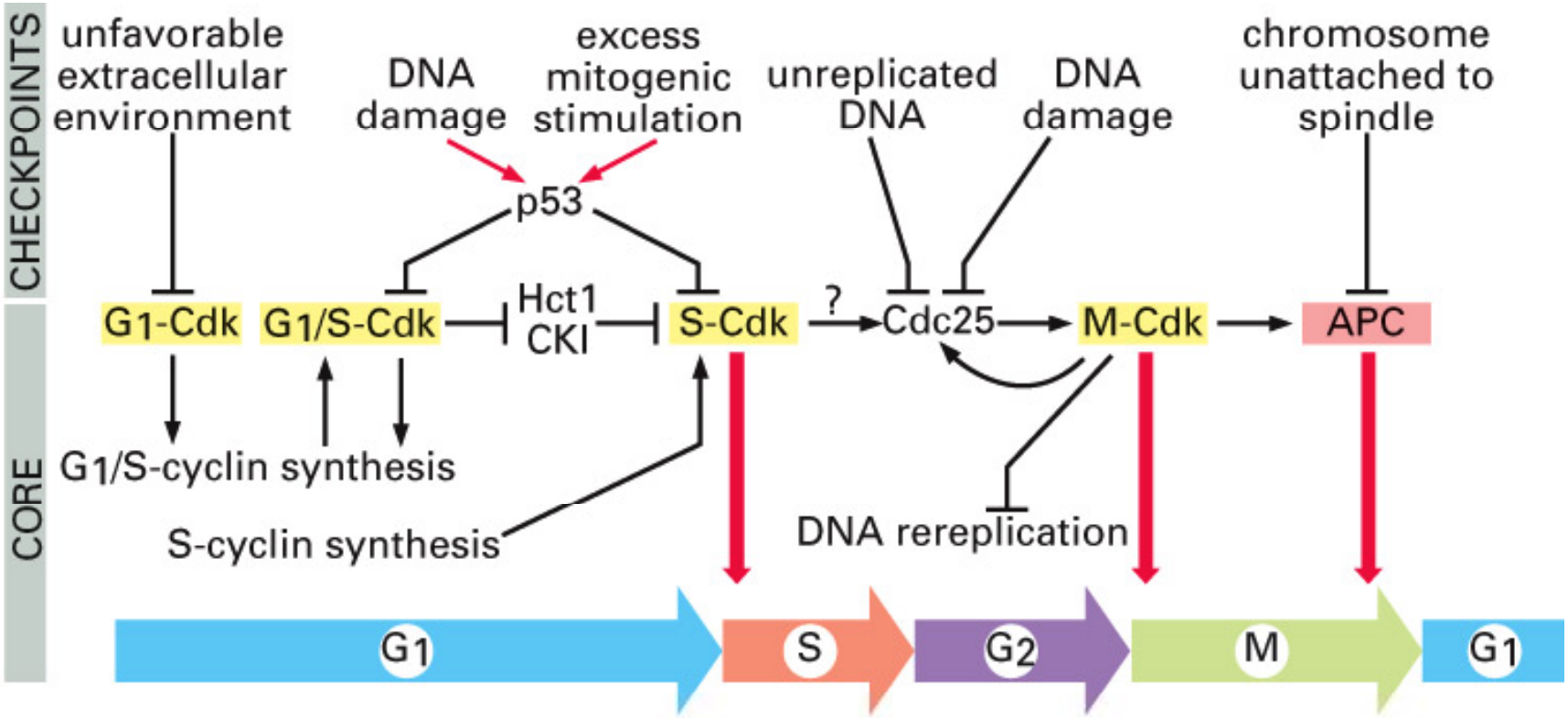


Figure 17–34. Molecular Biology of the Cell, 4th Edition.

Table 9.3 Apoptosis versus necrosis

	Apoptosis	Necrosis
Provoking stimuli		
	programmed tissue remodeling	metabolic stresses
	maintenance of cell pool size	absence of nutrients
	genomic damage	changes in pH, temperature
	metabolic derangement	hypoxia, anoxia
	hypoxia	
	imbalances in signaling pathways	
Morphological changes		
Affected cells	individual cells	groups of cells
Cell volume	decreased	increased
Chromatin	condensed	fragmented
Lysosomes	unaffected	abnormal
Mitochondria	morphologically normal initially	morphologically aberrant
Inflammatory response	none	marked
Cell fate	apoptotic bodies consumed by neighboring cells	lysis
Molecular changes		
Gene activity	required for program	not needed
Chromosomal DNA	cleaved at specific sites	random cleavage
Intracellular calcium	increased	unaffected
Ion pumps	continue to function	lost

Adapted from R.J.B. King, Cancer Biology, 2nd ed. Harlow, UK: Pearson Education, 2000.

Apoptosis

A decrease in programmed cell death (apoptosis) will favor the expansion of a cancer cell population. This can be achieved by a decrease in pro-apoptotic factors and/ or an increase in anti-apoptotic factors. Many slowly proliferating malignancies such as chronic lymphocytic leukemia, multiple myelomas, and colon and breast cancers over-express the antiapoptotic proteins Bcl-2 and Bcl-X_L.

Survivin

Survivin is a bifunctional protein that has a critical role in the regulation of both cell division and survival. Survivin is a member of the inhibitor of apoptosis family of proteins. These molecules act as suppressors of caspases, the effector enzymes of apoptosis. Survivin affects multiple signaling networks implicated in the regulation of apoptosis including the mitochondrial pathway of cell death, modulation of p53 checkpoints, and control of spindle formation and proper kinetochore attachment during cell division.

Several clinical trials targeting survivin are underway including immunotherapy or small-molecule antagonists.

References: D.C. Altieri. Targeted therapy by disabling crossroad signaling networks: the survivin paradigm. Mol. Cancer Ther. 5: 478-482, 2006.

Ambion TechNotes Newsletter 13, 7-11, 2007.

DNA methylation

DNA methylation in eukaryotes involves addition of a methyl group to the 5 position of a cytosine ring. DNA methylation is often associated with the silencing of gene transcription. Genes that may be silenced in cancer cells by methylation include tumor suppressor genes, genes that suppress tumor invasion and metastasis and DNA repair genes. 5-azacytidine, deoxyazacytidine and zebularine are compounds that block DNA methylation and they have shown promise in vitro and in clinical trials in leukemias.

Table 3-3 Induction of Differentiation in Culture

Stem Cell	Differentiation Markers	Inducers
Preadipocyte	Adipocyte	Insulin, cort, cell density
Basal keratinocyte	Cornified envelope	RA deficiency, cell density
Myoblast	Myotube	GF deficiency, cell density
Squamous cell carcinoma	Cornified envelope	GF deficiency, cort
Embryonal carcinoma	Endoderm, mesoderm, ectoderm	RA, ara-C, mito, HMBA, coculture with blastocyst
Neuroblastoma	Neuron, neurotransmitter, action potential	PI, 6TG, ara-C, MTX, dox, bleo, RA, GF deficiency
Melanoma	Dendrite, melanin, tyrosinase	PI, dox, DMSO, TPA, RA, MSH
Colon adenocarcinoma	Mucus, dome formation, CEA, columnar cell	NMF, DMSO, butyrate, low glucose, IFN, HMBA, cell density
Breast adenocarcinoma	Casein, dome formation	RA, PGE, DMSO
Bladder transitional cell carcinoma	Keratin filament, loss of surface antigen	HMBA
Erythroleukemia	Mature erythroid cell, hemoglobin	Dox, ara-C, 6TG, mito, dact, aza, hemin, DMSO, HMBA, CSF, RA, IFN
Promyelocytic	Granulocyte, macrophage	IFN, CSF, vitD, TPA, DMSO, NMF, dact, HMBA, aza, ara-C, RA
Myelocytic leukemia	Granulocyte, macrophage	CSF, RA, vitD, ara-C, dact, DMSO, TPA, cort, dox

ara-C = cytarabine; aza = 5-azacytidine; bleo = bleomycin; CEA = carcinoembryonic antigen; cort = glucocorticoids; CSF = colony-stimulating factor; dact = dactinomycin; DMSO = dimethylsulfoxide; dox = doxorubicin; GF = growth factor; hemin; HMBA = hexamethylbisacetamide; IFN = interferon-a or -g; mito = mitomycin C; MSH = melanocyte-stimulating hormone; MTX = methotrexate; NMF = N,N-dimethylformamide; PGE = prostaglandin E; PI = phosphodiesterase inhibitor; RA = retinoic acid; TPA = 12-0-tetradecanoylphorbol-13-acetate; vitD = 1,25-dihydroxy vitamin D; 6TG = 6-thioguanine. (Adapted from Reiss M et al,[178] Waxman S et al,[179] and Cheson BD et

TELOMERASE

The ends of linear chromosomes in eukaryotes are known as telomeres. They contain tandem repeated sequences which in humans may be TTAGGG

In the replication of DNA, after removal of the RNA primer at the 5’ end of a strand by RNAseH activity, conventional DNA polymerases can not fill in the gap. This problem can be solved by the telomerase enzyme. Telomerase consists of RNA and protein. The RNA hybridizes with the 3’ end of the DNA duplex and serves as a template for extension of the 3’ end. It does this in a repetitive manner to provide a sufficient extension for an RNA primer to be added that is complementary to the 3’ end. DNA polymerase can then fill the gap from the 5’ end. The eventual loss of the RNA primer is compensated by the telomerase catalyzed extension.

Without telomerase activity there will be a progressive loss of DNA at the end of the chromosome.

TELOMERASE

Telomerase activity is found in embryonic tissues and in germ cells and some adult tissues that have high rates of division including thymus and intestine. It is not found in most adult tissues but has been detected in many types of tumor and a variety of human cancer cell lines.

It has been suggested that telomerase activity may be an important factor in the immortalization of cancer cell lines.

The reproductive cell death induced by ionizing radiation in cancer cells has been shown to be accompanied by a decrease in telomerase activity. Detection of telomerase activity has been proposed as a diagnostic procedure for cancer tissue including pancreatic cancer.

Reference: Robert Weinberg, The Biology of Cancer, 2007, pages 368-398.

TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE

Unlike other DNA polymerases, terminal deoxynucleotidyl transferase does not require a template strand for DNA synthesis. It adds a single strand DNA sequence. Terminal deoxynucleotidyl transferase activity is normally found only in the precursor cells for lymphocytes in bone marrow. The enzyme is believed to have a role in immune function. Terminal deoxynucleotidyl transferase can serve as a diagnostic marker for circulating leukemic cells.

DNA REPAIR

1. nucleotide excision repair

Xeroderma pigmentosum can be caused by a defect in the excinuclease that cleaves the DNA near a pyrimidine dimer. There is a very high risk of skin cancer with this hereditary condition.

2. base excision repair

In this mechanism repair is initiated by a purine or pyrimidine glycosylase.

3. mismatch repair

Hereditary nonpolyposis colon cancer (HNCC) may affect 1 in 200 people). It results from defects in mismatch repair. In most cases there are mutations in one of two genes, hMSH2 and hMLH1). These are the human equivalents of MutS and MutL of E. coli. hMSH2 binds at a mismatched DNA base pair and hMLH1 participates in cleaving DNA near the mismatch to initiate the repair process.

4. O6 alkylguanine alkyl transferase

This protein removes small alkyl groups such as methyl groups from the O6 position of guanine residues.

ATM

Ataxia telangiectasia is caused by mutations in the ATM gene encoding a protein kinase that is activated by double stand DNA breaks. ATM kinase activity initiates a phosphorylation cascade that modifies substrates controlling cell cycle arrest and DNA repair.

Ataxia telangiectasia has an autosomal recessive inheritance and is characterized by progressive neurodegeneration, immunodeficiency and a high predisposition to the development of lymphoid malignancies.

STATS and oncogenic signaling pathways

STAT (Signal transducer and activator of transcription) family proteins are latent transcription factors that convey signals from cytokine and growth-factor receptors to the nucleus.

STAT proteins, particularly STAT3 and STAT5 proteins are frequently over-activated in a variety of human solid tumors and blood malignancies.

Persistent STAT3 signaling promotes the growth and survival of cancer cells and induces angiogenesis.

Tumor cells that become dependent on persistent STAT3 signaling are more sensitive to STAT3 inhibitors than normal cells. Small molecule inhibitors of STAT3 and STAT 5 are being investigated e.g. WP-1034.

Reference:H. Yu and R. Jove. The STATS of cancer - new molecular targets come of age. Nature Reviews Cancer 4: 97-105, 2004

GROWTH FACTORS

Insulin-like growth factor-I (IGF-I) levels are known to be decreased by caloric restriction. Supplementation with IGF-I was shown to prevent the protective effect of caloric restriction on tumor progression in p53 deficient mice.

(Dunn et al., Cancer Res., 57:4667, 1997).

The loss of TGF-beta receptor gene in fibroblasts of a knockout mouse resulted in neoplasia in the prostate and forestomach associated with an abundance of stromal cells. The occurrence of transformation in the adjacent epithelial cells was accompanied by activation of hepatocyte growth factor signaling.

(Bhowmick et al., Science 303: 848-851, 2004)

Table 5.1 Growth factors (GFs) and tyrosine kinase receptors that are often involved in tumor pathogenesis

Name of GF	Name of receptor	Cells responding to GF
PDGF[a]	PDGF-R	endothelial, VSMCs, fibroblasts, other mesenchymal cells, glial cells
EGF[b]	EGF-R[c]	many types of epithelial cells, some mesenchymal cells
NGF	Trk	neurons
FGF[d]	FGF-R[e]	endothelial, fibroblasts, other mesenchymal cells, VSMCs, neuroectodermal cells
HGF/SF	Met	various epithelial cells
VEGF[f]	VEGF-R[g]	endothelial cells in capillaries, lymph ducts
IGF[h]	IGF-R1	wide variety of cell types
GDNF	Ret	neuroectodermal cells
SCF	Kit	hematopoietic, mesenchymal cells

[a]PDGF is represented by four distinct polypeptides, PDGF-A, -B, -C, and -D. The PDGF-Rs consist of at least two distinct species, α and β, that can homodimerize or heterodimerize and associate with these ligands in different ways.
[b]The EGF family of ligands, all of which bind to the EGF-R (ErbB1) and/or heterodimers of erbB1 and one of its related receptors (footnote c), includes—in addition to EGF—TGF-α, HB-EGF, amphiregulin, betacellulin, and epiregulin.
[c]The EGF-R family of receptors consists of four distinct proteins, ErbB1 (EGF-R), ErbB2 (HER2, Neu), ErbB3 (HER3), and ErbB4 (HER4). They often bind ligands as heterodimeric receptors, for example, ErbB1 + ErbB3, ErbB1 + ErbB2, or ErbB2 + ErbB4; ErbB3 is devoid of kinase activity and is phosphorylated by ErbB2 when the two form heterodimers. ErbB3 and ErbB4 bind neuregulins, a family of more than 15 ligands that are generated by alternative splicing. Because ErbB3 has no intrinsic kinase activity, it becomes phosphorylated in heterodimeric complexes by ErbB2, which has no ligand of its own but does have strong tyrosine kinase activity.
[d]FGFs constitute a large family of GFs. The prototypes are acidic FGF (aFGF) and basic FGF (bFGF); in addition there are other known members of this family.
[e]There are four well-characterized FGF-Rs.
[f]There are four known VEGFs. VEGF-A and -B are involved in angiogenesis, while VEGF-C and -D are involved predominantly in lymphangiogenesis.
[g]There are three known VEGF-Rs: VEGF-R1 (also known as Flt-1) and VEGF-R2 (also known as Flk-1/KDR), involved in angiogenesis; and VEGF-R3, involved in lymphangiogenesis.
[h]The two known IGFs, IGF-1 and IGF-2, both related in structure to insulin, stimulate cell growth (i.e., increase in size) and survival; they also appear to be mitogenic.

Abbreviation: VSMC, vascular smooth muscle cell.

Adapted in part from B. Alberts et al., Molecular Biology of the Cell, 4th ed. New York: Garland Science, 2002.

Kaposin B

Kaposi's Sarcoma-associated Herpes Virus (KSHV) has been linked to the formation of Kaposi's sarcoma in which there is a proliferation of spindle-shaped endothelial cells. One of the gene products of KSHV is kaposin B. This protein activates the p38/MK2 pathway and results in the stabilization of mRNAs for cytokines including IL-6.

- (McCormick and Ganem, Science 307: 739-741, 2005)

Growth and the Cell Cycle in Cancer -Suggested Reading

M. Andreeff, D.W. Goodrich and H.P. Koeffler, In Holland-Frei Cancer Medicine - 8th ed., Part II, Section I, 3. Cell Proliferation and Differentiation (2010).

J.C. Reed, In Holland-Frei Cancer Medicine - 8th ed., Part II, Section I, 4. Apoptosis and Cancer (2010).

S.A. Aaronson, In Holland-Frei Cancer Medicine - 8th ed., Part II, Section I, 5. Growth Factors and Signal Transduction in Cancer (2010).

R. Weinberg, The Biology of Cancer, Garland Press, Chapter 8, 9 and 10, (2007).