25

CANCER

REVIEW THE CONCEPTS

1. Cancer cells generally have lost the regulation that governs cell physiology in normal cells. This loss of regulation can occur in one or more of the following areas:
   - Transduction of growth signals to the nucleus
   - Gene expression
   - Cell cycle control
   - DNA replication and repair

   In addition, cancer cells can develop aggressive proliferative properties that differentiate them from normal cells including:
   - Ability to stimulate angiogenesis
   - Ability to metastasize (via EMT)
   - Rewiring of of glucose metabolism - Warburg effect

2. Benign tumors remain localized to the tissue of origin, often maintaining normal morphology and function, and are pathological only if their sheer mass interferes with tissue function or if they overproduce a hormone or other factor that disrupts normal body homeostasis. Malignant tumors possess cells that divide more rapidly than normal, fail to die by apoptosis, invade surrounding tissues, and may metastasize to other parts of the body. The genetic difference between benign colon polyps and malignant colon carcinoma is in the number of cancer-promoting mutations. The polyp possesses a loss-of-function mutation in the *APC* gene, whereas the malignant carcinoma possesses the APC mutation as well as other cancer-promoting mutations in the *K-ras* and *p53* genes.

3. Otto Warburg discovered that energy metabolism in cancer cells differs substantially from that in normal cells. In contrast to normal cells, most cancer cells rely on glycolysis for energy production irrespective of whether oxygen levels are high or low, producing large amounts of lactate. The metabolism of glucose to lactate generates only 2 ATP molecules per molecule of glucose, in contrast to oxidative phosphorylation, which can generate up to 36 molecules of ATP per molecule of glucose. The use of glycolysis to produce energy even in the presence of oxygen, called aerobic glycolysis, was first discovered in cancer cells by Warburg and is therefore called the "Warburg effect."

4. The growth factors βFGF, TGFα, and VEGF all promote angiogenesis, the proliferation of blood vessels. If cancer cells acquire the ability to induce angiogenesis, then the tumor can develop its own vasculature and grow to a virtually unlimited size.

5. Metastasis is the process by which cancer cells escape their tissue of origin, travel through the circulation, and invade and proliferate within another tissue or organ.
   a. Batimastat inhibits enzymes that degrade the extracellular matrix, and thus cancer cells will be unable to digest the basement membrane and escape the tissue of origin.
6. The epithelial to mesenchymal transition (EMT) is thought to play a crucial role during the process of metastasis in certain cancers. During normal development, the conversion of epithelial cells into mesenchymal cells is a step in the formation of some organs and tissues. An EMT requires distinct changes in patterns of gene expression and results in fundamental changes in cell morphology, such as loss of cell-cell adhesion, loss of cell polarity, and the acquisition of migratory and invasive properties. During metastasis, the EMT regulatory pathways are thought to be activated at the invasive front of tumors, producing single migra-tory cells.

7. The increased incidence of cancer with age is explained by a "multi-hit" model; successive mutations or alterations in gene expression correspond to the discrete stages leading to a lethal tumor. For example, many colon cancers contain mutations in *APC, p53,* tumor-suppressor genes, and in *ras*. The APC mutation is found in polyps, an early stage of colon cancer, while *p53* mutation is required for malignancy. In mice, overexpression of *myc* or expression of *rasD* causes can-cer only after a long lag. However, these two genes act synergistically to cause cancer in at least one-third the time of either alone.

8. Proto-oncogenes are genes that become oncogenes by mutations that render them constitutively or excessively active. They promote cell growth, inhibit cell death, or promote some other aspect of the cancer phenotype such as metastasis. Tumor-suppressing genes restrain growth, promote apoptosis, or inhibit some

other aspect of the cancer phenotype. Gain of function mutations convert proto-oncogenes to oncogenes, and thus only a single copy of the proto-oncogene needs to be mutated to an oncogene to be cancer promoting. Loss-of-function mutations in tumor-suppressor genes are cancer promoting, and thus both copies of the gene usually need to be inactivated unless mutation of a single copy functions in a dominant negative manner as is the case with some mutations in the *p53* gene. The *ras,* BCL-2, MDM2, and JUN genes are proto-oncogenes. The *p53* and *p16* genes are tumor-suppressor genes.

9. Inactivation of genome maintenance factors will cause an increase in the incidence of mutations in the genome. This can lead to oncogenic mutations that can transform cells.

10. In hereditary retinoblastoma, individuals have inherited one mutated copy of the *RB* gene, and therefore require only a spontaneous mutation in the other copy to lack functional Rb protein. The relative frequency of a single spontaneous mutation is high enough that these individuals develop retinoblastoma early in life in both of their eyes. However, in spontaneous retinoblastoma, individuals have inherited two normal copies of the *RB* gene. Therefore, spontaneous mutations in each copy of *RB* must occur within a single cell for it to lack functional Rb. The likelihood of a cell's possessing both mutations is extremely low, and thus these mutations rarely occur until adulthood and then usually in a single eye. Because the chance of an individual with hereditary retinoblastoma receiving an inactivating mutation in the other copy of the *RB* gene in any one of the susceptible cells is quite high, the disease is inherited in a dominant manner.

11. Many individuals are genetically predisposed to cancer because of the loss or inactivation of one copy of a tumor-suppressor gene. Loss-of-heterozygosity (LOH) describes the loss or inactivation of the second, normal copy in a somatic cell, a prerequisite for the development of a tumor because one functional copy of a tumor-suppressor gene is usually sufficientfornormalfunction.Sincethe development of cancer requires loss-of-function in one or more tumor-suppressor genes (e.g., *RB, p53*), LOH of at least one allele is found in virtually all malignant tumors. One mechanism by which loss-of-heterozygosity develops is the missegregation of chromosomes during mitosis. The spindle assembly checkpoint normally arrests cells in mitosis until chromosomes are properly aligned on the mitotic spindle. If this checkpoint is not functional, missegregation events leading to LOH are more frequent.

12. Transmembrane growth factor receptors such as the EGF receptor are protein tyrosine kinases. Cytokine receptors such as the erythropoietin receptor activate associated JAK kinases. In the case of the EGF receptor, once activated, these receptors dimerize and activate a series of signal transduction events that ultimately result in changes in gene expression. As the EGF receptor is a tyrosine kinase, it catalyzes the phosphorylation of protein substrates once activated. A point mutation in the transmembrane region of the HER2 receptor causes receptor dimerization and subsequent activation of the receptor's tyrosine kinase properties, even in the absence of EGF ligand. The result is a constitu-tively active HER2 receptor.

13. Gain-of-function (GOF) mutations in the *RAS* gene (i.e., *rasD*), renders Ras consti-tutively active in the GTP-bound form. Constitutively active Ras activates the growth-promoting MAPK signaling pathway, even in the absence of upstream signals from growth factor-bound receptor tyrosine kinases. Loss-of-function mutations (LOF) in *NF1* have the same effect as GOF mutations in *RAS* because *NF1* encodes a protein that hydrolyses GTP bound to Ras, converting Ras to the inactive, GDP-bound form. Since GOF mutations (such as the formation of RasD) require only a single allele to be mutated, whereas in LOF mutations
(such as the inactivation of NF1) usually both alleles must be mutated, cancer-promoting mutations in *ras* are more common than cancer-promoting mutations in *NF1*.

14. In Burkitt's lymphoma, translocation places the *c-MYC* gene under the infl uence of the antibody heavy-chain gene-enhancers. Thus, myc is expressed at high levels, but only in cells in which antibodies are produced (e.g., B-lymphocytes). Thus, this mutation is found in lymphomas rather than in other types of cancers. myc can also be rendered oncogenic by amplifi cation of a DNA segment containing the *MYC* gene. This type of mutation is not restricted to lymphomas.

15. Smad4 is a transcription factor that transduces the signal generated when TGFβ binds to its receptor on the plasma membrane. Smad4 promotes expression of the *p15* gene, which, like *p16,* inhibits cyclin D-CDK function, promoting cell cycle arrest in G_1. Smad4 also promotes expression of extracellular matrix genes and plasminogen activator inhibitor 1 (PAI-1), both of which inhibit the metastasis of tumor cells. Thus, a loss of Smad4 abrogates both the proliferation and metastasis inhibiting effects of TGFβ signaling.

16. The INK4 inhibitor normally acts as a tumor suppressor, and loss of INK4 mimics overproduction of cyclin D1. The INK4 locus encodes at least three tumor-suppressor genes, making it the most highly vulnerable locus in the human genome. In addition to harboring the p16-encoding gene *INK4a,* immediately upstream is the INK4b locus, which encodes p15, another cyclin D–CDK4/6 inhibitor. In addition to these CDK inhibitors the locus also codes for p14ARF, a key activator of the tumor suppressor p53.

17. Epigenetic changes, such as changes in DNA methylation, histone modification, and miRNA levels, can contribute to tumorigenesis by dysregulating the expression pf tumor suppressors or oncogenes..One example of how epigenetic changes may contribute to tumorigenesis is DNA methylation. Hypermethylation of CpG islands at tumor-suppressor genes switches off these genes, whereas global hypomethylation leads to genome instability and inappropriate activation of oncogenes and transposable elements.

18. E7 binds to Rb and inactivates it.

19. p53 inhibits malignancy in multiple ways. When cells are exposed to ionizing radiation, p53 becomes stabilized and functions as a transcription factor to promote expression of $p21^{CIP}$—leading to cell-cycle arrest in G_1—and to repress expression of cyclin B and topoisomerase II, leading to cell-cycle arrest in G_2. Thus, p53 functions in DNA damage checkpoints during both G_1 and G_2 of the cell cycle. p53 can also promote apoptosis, in part by promoting transcription of Bax . A loss of cell-cycle checkpoints and apoptosis are both characteristics of cancer cells. The carcinogen benzo(a)pyrene is activated by enzymes in the liver to become a mutagen that coverts guanine to thymine bases, including several guanines in p53, rendering the gene nonfunctional.

20. p53 is a tetramer of four identical subunits that acts as a transcription factor to regulate cell proliferation by inducing cell cycle arrest or apoptosis in response to a number of signals. p53 tetramers that contain at least one subunit with an inactivating missense mutation would have reduced ability to activate transcription. Cells with this reduced p53 function would exhibit excessive proliferation and loss of cell cycle arrest or apoptosis, and so would be selected for during cancer progression, due to a proliferative growth advantage. However, the loss of function is incomplete and in order to proliferate more rapidly tumor cells often lose the remaining functional allele.

21. Protein-coding sequences make up about one percent of the genome. Only these mutations would cause production in neo-antigens. In addition, mutations that affect regulatory sequences, gene amplifications or deletions, and multiple other mutations that do affect gene function would not generate a neo-antigen.

22. The antibody part of the chimeric receptor is engineered to recognize tumor-specific antigens on the surface of tumor cells (CD19 in B-cell cancer cells, for example). The antigen-binding VH and VL domains of a monoclonal antibody are used.
These antibody domains are linked to the cytosolic domain of the T-cell receptor ζ subunit. The binding to the antibody domains triggers the activation by the T-cell receptor domain of the ZAP70 protein tyrosine kinase. This activates signal 1, the signal transduction pathway in a T cell that activates its ability to kill the antigen presenting cell. In addition, the chimeric antigen receptor includes co-stimulatory receptors, such as CD28 or 4-1BB, which were found necessary for the T-cells to actually kill the targeted tumor cells.