25 Cancer

Review the Concepts

1. 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.

2. 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.

2a. 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.

2b. Inhibition of integrin function prevents attachment of cancer cells to the basement membrane, an early step in metastasis.

2c. Bisphosphonate inhibits osteoclasts, which are recruited and activated by many cancer cells, particularly cancers that originate in bone marrow; the cancer cells will not be able to escape from surrounding bone tissue to enter the circulation and metastasize or populate other bone marrow tissues.

3. The growth factors bFGF, 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.

4. Mouse 3T3 cells normally grow in a monolayer. In a transformation assay, the 3T3 cells are transfected with DNA fragments from a human tumor. If any of the cells pick up and express a *ras* gene from the tumor DNA, they lose their contact inhibition and form a focus, a pile of cells that can be seen under the microscope. Thus only the cells that give rise to foci contain a *ras* gene from the human tumor.

   In normal mouse fibroblast cells, p53 would protect the cells against transformation. But, the 3T3 cells have loss-of-function mutations in either p53 or p19ARF.

5. 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*, *DCC*, and *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 ras^D causes cancer only after a long lag. However, these two genes act synergistically to cause cancer in at least one-third the time of either alone.

6. 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-suppressor 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,* and *jun* genes are proto-oncogenes. The *p53* and *p16* genes are tumor-suppressor genes.

7. DNA microarray analysis can identify differences in gene-expression patterns. To do this kind of comparison for different types of lymphoma, messenger RNA was extracted from many different patients. A DNA microarray can simultaneously determine the transcription of about 18,000 genes. For the lymphoma samples, a cluster diagram of gene expression revealed different groups, with similar expression patterns within each group.

8. 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.

9. 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 since one functional copy of a tumor-suppressor gene is usually sufficient for normal function. Since the 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 mis-segregation 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, mis-segregation events leading to LOH are more frequent.

10. Transmembrane growth factor receptors such as the EGF receptor are protein tyrosine kinases. Cytokine receptors such as the erythropoietin receptor activate associated JAK kinases.

10a. gp55 binds to the erythropoietin receptor, causing the receptor to dimerize and become activated, leading to the constitutive activation of associated JAK kinases, even in the absence of erythropoietin.

10b. The chimeric protein generated when the extracellular domain of the Trk receptor fuses with the N-terminal region of nonmuscle tropomyosin can dimerize due to the coiled-coil structure of the tropomyosin region. The Trk receptor tyrosine kinase is thus constitutively active even in the absence of ligand.

10c. A point mutation in the Her2 receptor likewise causes receptor tyrosine kinase dimerization and activation even in the absence of EGF ligand. The resulting constitutively active receptor is called the Neu oncoprotein.

11. Gain-of-function (GOF) mutations in the *ras* gene (i.e., ras^{D}), renders Ras constitutively 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 *NF-1* have the same effect as GOF mutations in

ras since *NF-1* 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 Ras^D) require only a single allele to be mutated, whereas in LOF mutations (such as the inactivation of NF-1) usually both alleles must be mutated, cancer-promoting mutations in *ras* are more common than cancer-promoting mutations in *NF-1*.

12. The v-Src protein lacks the carboxy-terminal 18 amino acids, including tyrosine 527. Phosphorylation of tyrosine 527 on c-Src by Csk causes a conformational change that inactivates Src. Because v-Src lacks this phosphorylation site, it is insensitive to Csk and therefore constitutively active.

13. In Burkitt's lymphoma, translocation places the *c-myc* gene under the influence 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 amplification of a DNA segment containing the *myc* gene. This type of mutation is not restricted to lymphomas.

14. 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.

15. The three viral proteins are E5, E6, and E7. E5 binds to PDGF receptor proteins and causes them to aggregate in the plasma membrane, which stimulates activation even in the absence of the normal growth-factor signal. E6 binds to p53 and accelerates its degradation. E7 binds to Rb and inactivates it.

16. 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.

17. In humans, normally only germ cells and stem cells possess telomerase activity. Telomerase maintains telomere ends and promotes immortality of cells, one characteristic of cancer. Since stem cells express telomerase, they may have a greater likelihood of becoming malignant, a concern that needs to be addressed if stem cells are to be used therapeutically to treat human disease.

Analyze the Data

a. Each of the three drugs causes the NSCLC cells to undergo apoptosis at a significant level. Thus the rationale for using these drugs is that they would induce the death of the cancer cells. However, nicotine appears to interfere with this induction of apoptosis, perhaps explaining why NSCLC is resistant to chemotherapy.

b. PARP is cleaved upon treatment with the four chemotherapeutic drugs but is not cleaved when nicotine is also present. Because PARP is cleaved upon apoptosis, these data suggest, as in (a), that the chemotherapeutic drugs induced apoptosis, but that this apoptosis does not occur if nicotine is present. p53 and p21 both appear to be produced more, or stabilized, in cells treated with the chemotherapeutic drugs. Each of these proteins would contribute to arresting the cells in G_1 or G_2,

thereby keeping them from proliferating. Such an increase in the amounts of these two proteins does not occur when nicotine is also present. Thus, nicotine circumvents the ability of the chemotherapeutic drugs to induce the cells to arrest in G_1 or G_2 (which then likely leads to apoptosis). The fact that actin levels are unaffected by the various treatments indicates that the chemotherapeutic drugs specifically affect the cell cycle and nicotine interferes with this effect. Moreover, the actin data serve to ensure that the gel lanes have been loaded equally and that the differences observed in the amount of other proteins from lane to lane is biologically relevant and not a loading artifact.

c. Nicotine causes the levels of XIAP and Survivin proteins to increase (see the blot on text page 1147), and this increase is required for the survival of the cells in the presence of the chemotherapeutic drugs (the graph below shows that treatment with siRNA to prevent XIAP and Survivin increases causes cells to undergo gemcitabine-induced apoptosis even when nicotine is present). The protein level increases appear to be mediated by the PI-3 kinase pathway, because inhibition of this pathway with LY294002 eliminates the protein increases and causes apoptosis, as assessed by PARP cleavage. A likely signaling pathway is that nicotine binds to a cell-surface receptor that activates PI-3 kinase which, in turn, leads to increased Survivin and XIAP, both of which help protect the cells against the apoptosis induced by chemotherapeutic drugs.

d. The patients who continue to smoke will inhale nicotine that will bind to receptors and thus activate PI-3K. PI-3K activity will result in increased Survivin and XIAP, which will oppose the action of the chemotherapeutic drugs. Normally the drugs cause the cells to undergo apoptosis, thus destroying the tumor as well as some other cells. Thus chemotherapy will be essentially without benefit in individuals where nicotine prevents tumor cell death.