

Genetics and Genomics in Medicine Chapter 10

Questions & Answers

Multiple Choice Questions

Question 10.1

Which, if any, of the following statements is false?

- a) The p53 tumor suppressor regulates the G₂-M transition in the cell cycle by inhibiting the CDK2-cyclin E complex.
- b) p53 regulates the CDK2-cyclin E complex by stimulating the p21 tumor suppressor.
- c) The CDK2-cyclin E complex relies on stimulation by the E2F transcription factor.
- d) The RB1 protein normally binds the E2F transcription factor to prevent it working and so acts as a brake on the cell cycle

Answer 10.1

- a) The p53 tumor suppressor regulates the G₂-M transition in the cell cycle by inhibiting the CDK2-cyclin E complex.

Explanation 10.1

p53 regulates the G₁-S transition in the cell cycle.

Question 10.2

Which, if any, of the following statements is false?

- a) p53 acts as a brake on the cell cycle.
- b) p53 and RB1 are mutually antagonistic.
- c) p53 is normally inhibited by being bound to the MDM2 protein, but when phosphorylated, it changes conformation and is released from MDM2.
- d) At high concentrations p53 stimulates the transcription of various genes that inhibit apoptosis.

Answer 10.2

- b) p53 and RB1 are mutually antagonistic.
- d) At high concentrations p53 stimulates the transcription of various genes that inhibit apoptosis.

Question 10.3

Which, if any, of the following statements is false?

- a) p53 acts as a brake on cell growth but stimulates apoptotic pathways.
- b) apoptosis occurs only after a death signal is received by one cell from another cell.
- c) the death signal starts a pathway that culminates in the activation of a class of proteolytic enzymes called caspases.
- d) caspases attack cellular proteins and release an endonuclease that cleaves the cellular NA into small fragments.

Answer 10.3

- b) apoptosis occurs only after a death signal is received by one cell from another cell.

Explanation 10.3

There are also intrinsic apoptosis pathways that respond to internal cellular damage that can be caused by reactive oxygen species, ionizing radiation and so on.

Question 10.4

Which, if any, of the following statements is false?

- a) Chromothripsis is an extraordinary event in which multiple chromosomes within a cell are shattered into many pieces.
- b) Chromothripsis is much more like to occur in cells where the *TP53* gene has received loss-of-function mutations.
- c) Kataegis is a type of somatic hypermutation.
- d) The mutations involved in a kataegis event are clustered in one subchromosomal region.

Answer 10.4

- a) Chromothripsis is an extraordinary event in which multiple chromosomes within a cell are shattered into many pieces.

Explanation 10.4

Chromothripsis is a localized event. Shattering of multiple chromosomes would leave a cell so adversely affected that it could not be expected to function normally, or even survive.

Question 10.5

Which, if any, of the following statements is false?

- a) Conventional chromosome banding analyses are often very difficult to carry out in the case of solid tumor samples.
- b) Spectral karyotyping simply means standard chromosome FISH that is applied to tumor cells.
- c) Genomewide analyses can be carried out by microarray hybridization to look for evidence of many types of chromosome abnormalities in solid tumors.

- d) Genomewide analyses cannot be carried out by chromosome FISH analyses on cancer cells.

Answer 10.5

- b) Spectral karyotyping simply means standard chromosome FISH that is applied to tumor cells.
- d) Genomewide analyses cannot be carried out by chromosome FISH analyses on cancer cells.

Explanation 10.5

Spectral karyotyping (SKY) is an exceptional type of multicolour chromosome FISH in which genomewide analyses are carried out to get a detailed representation of all chromosomes in cancer cells (see Figure 10.14 on page 403).

Question 10.6

Which, if any, of the following statements is false?

- a) Mismatch repair is dedicated to repairing errors made during DNA replication.
- b) MutS α is responsible for recognizing single base mismatches during DNA replication.
- c) MutS β is responsible for recognizing all short insertion and deletion errors made during DNA replication.
- d) MutL α contributes an endonuclease activity that is required to nick the DNA during DNA repair.

Answer 10.6

- c) MutS β is responsible for recognizing all short insertion and deletion errors made during DNA replication.

Explanation 10.6

MutS α recognizes single nucleotide insertions/deletions as well as single base mismatches while MutS β recognizes longer insertion/deletion errors that arise from replication slippage.

Question 10.7

Which, if any, of the following statements is incorrect or very likely to be incorrect?

- a) Epigenetic dysregulation is essentially a universal feature of tumors.
- b) Epigenetic dysregulation in cancer cells always arises as a result of mutation at a chromatin modifier locus.
- c) There is an overall increase in DNA methylation across the genome of cancer cells but with local DNA hypomethylation at the promoters of a few hundred genes.
- d) Epigenetic dysregulation may sometimes initiate tumorigenesis.

Answer 10.7

- b) Epigenetic dysregulation in cancer cells always arises as a result of mutation at a chromatin modifier locus.
- c) There is an overall increase in DNA methylation across the genome of cancer cells but with local DNA hypomethylation at the promoters of a few hundred genes.

Explanation 10.7

In addition to mutation at a chromatin modifier locus, increasing evidence supports epigenetic dysregulation as an occasional initiator of tumorigenesis.) There is an overall reduction in DNA methylation across the genome of cancer cells but with local DNA hypermethylation at the promoters of a few hundred genes.

Fill in the Blanks Question

Question 10.8

Fill in the blanks below with single words.

_____1_____ is an important natural process that is devoted to eliminating diseased or potentially harmful deviant cells. There are two classes of pathways. In one of these neighboring cells deliver signals that are received by _____2_____ on the surface of those cells selected to undergo _____1_____. Other pathways, such as the mitochondrial _____1_____ pathway, respond to certain types of _____3_____ damage (such as that caused by harmful _____4_____ _____5_____ species or exposure to dangerous levels of _____6_____ radiation). In most cases the _____1_____ pathway ends by inducing the cell to produce a class of proteolytic enzymes known as _____7_____. _____7_____ are the cell's executioners: they inactivate all kinds of important proteins in the cell, and they release an _____8_____ that cleaves DNA into small fragments. Because each of our cells has the potential to commit suicide, the pathways of _____1_____ need to be tightly regulated.

Answer 10.8

1. apoptosis. 2. receptors. 3. oxidative 4. reactive. 5. oxygen. 6. ionizing. 7. caspases. 8. endonuclease.

Essay, Listing, and Matching Questions

Question 10.9

With reference to cancer spreading what is involved in intravasation and extravasation?

Answer 10.9

Cancer spreading involves both local invasion of adjacent tissues, and also dissemination through the bloodstream and the lymphatic system, in which case the cancer cells are described as metastatic cells.

To enter the blood stream from a tissue, cancer cells reduce adhesion to neighboring cells and then clear a path for migration into the vasculature- rich stroma. Once at the vasculature, cells can freely enter the bloodstream if the vasculature is discontinuous, as in certain regions of the liver, bone marrow, and kidneys. But *intravasation* is required if the vasculature is continuous. In the process of intravasation metastatic cells sometimes release compounds, such as vascular endothelial growth factor, that cause endothelial cells to retract so that the metastatic cells can squeeze between and past the endothelial cells. Alternatively, they induce endothelial cell death by releasing reactive oxygen species and factors including matrix metalloproteinases.

After passing through the bloodstream the cancer cells can leave the bloodstream to reach a secondary site. The process, called extravasation, is the reverse of intravasation but involves the same mechanisms: metastatic cells induce endothelial cell retraction or death as a way of breaching the endothelial cell barrier.

Question 10.10

Cancers develop as a result of natural selection operating at the cellular level. What is meant by this, and if there is strong selection pressure on cells to evolve into cancer cells, why do we not all succumb to cancer?

Answer 10.10

Cancer develops progressively when a series of successive mutations disrupt the normal controls that limit cell proliferation, or that induce apoptosis. Cells become able to break free from suppressive controls exerted by their neighbors in a tissue microenvironment. This is where natural selection at the cellular level is important: each successive mutation that disrupts normal controls on cellular proliferation and apoptosis confers an additional selective growth advantage on its descendants. As a result, there is strong selection pressure on cells to evolve through a series of stages into tumor cells.

Despite such strong selection pressure on cells to evolve into tumor cells, a minority of us succumb to cancer? Certainly, if we were to live long enough, cancer would be an inevitable consequence of random mutations. However, an opposing force of natural selection works at the

level of the organism (to keep us healthy and free from tumors—at least until we have produced and raised children). It involves different mechanisms, not least immunosurveillance to detect and kill cancer cells (individuals whose immune systems are suppressed are more susceptible to cancer).

Another factor is that natural selection working at the organismal level also operates over a much longer timescale than does the selection pressure in favor of tumor cell formation. Cancer cells can successfully proliferate and form tumors within an individual person, but they do not leave progeny beyond the life of their human host; tumorigenesis processes must start afresh in a new individual. But individuals who have efficient cancer defense mechanisms are able to pass on good anti-cancer defense genes to their offspring, and the anti-cancer defense systems continue to evolve from one generation to the next.

Question 10.11

As cancer cells evolve they acquire distinguishing biological characteristics. Describe five of them and for each give examples of how the biological capability can be acquired.

Answer 10.11

Any five from the following list

Self-sufficiency in growth signaling	Activate cellular oncogene
Insensitivity to signals suppressing growth	Inactivate the <i>TP53</i> tumor suppressor gene to avoid p53-mediated cell cycle arrest
Ability to avoid apoptosis	Produce insulin growth factor as a survival factor
Replicative immortality	Switch on telomerase
Genome instability	Inactivate certain genes involved in DNA repair
Induction of angiogenesis	Produce factor that induces vascular endothelial growth factor
Tissue invasion and metastasis	Inactivate E-cadherin
Ability to avoid immune destruction	Paralyze infiltrating cytotoxic T lymphocytes and natural killer cells by secreting an immunosuppressive factor such as transforming growth factor beta
Induction of tumor-promoting inflammation	Redirect inflammation-causing immune system cells that infiltrate the tumor so that they help in various tumor functions (see Table 10.3)
Reprogramming energy metabolism	Induce aerobic glycolysis

Question 10.12

Distinguish between driver mutations and passenger mutations in cancer. How many driver mutations might be expected in cancers?

Answer 10.12

As normal cells evolve to become cancer cells, they pick up many somatic changes—both genetic and epigenetic. A small subset of the genetic changes, known as driver mutations, result in altered expression of genes that confer a growth advantage to their descendants. They are positively selected and causally implicated in cancer development. The remainder are passenger mutations.

Whereas there can be many thousands of passenger mutations in a cell, the number of driver mutations per cell is small. Massively parallel genome sequencing studies have suggest that most breast cancers have 6 or less driver mutations to which must be added epigenetic changes such as silencing of tumor suppressor alleles (and also the possibility of tumor initiation). The number of driver mutations also depends on the type of tumor. Less driver mutations might be expected, for example, in tumors of blood cells (which are not constrained by being part of a solid tissue) and childhood tumors.

Question 10.13

As a cancer develops the mutation rate accelerates. How does that happen?

Answer 10.13

The average rate of mutation in human cells is low (about 10^{-6} per gene per cell) and the majority of cancer-causing mutations are recessive at the cellular level, so that both alleles need to be mutated. Cancer might therefore be expected to be highly improbable: the chance that any cell would receive successive mutations, often in both alleles, at several cancer-susceptibility loci would normally be vanishingly small. Cancer nevertheless is common, and altered expression at a few cancer- susceptibility loci can be sufficient. One major explanation is that early driver mutations greatly increase the probability of later mutations in two ways, as listed below.

- 1) The first way is by giving the cell a growth advantage. If cells with a driver mutation have an increased growth rate, they will produce more progeny than other cells and will produce an expanded target of mutant cells, thereby increasing the probability of a subsequent mutation (see Figure 10.4A).
- 2) The second, and most important, way of increasing the probability of later mutations in cancer is by destabilizing the genome. Chromosome instability is a feature of most tumor cells, producing grossly abnormal karyotypes with abnormal numbers of chromosomes and frequent structural arrangements that can activate oncogenes or cause a loss of tumor suppressor genes. In some cancers, a form of global DNA instability occurs—it is caused by mutations in key DNA repair genes and can result in greatly elevated mutation rates.

Mutations in genes that regulate epigenetic modifications result in additional epigenetic instability that can result in altered expression at cancer-susceptibility loci. Additionally, some types of epigenetic change cause genome instability.

Question 10.14

Tumors gradually acquire mutations to evolve from benign to malignant lesions. Because that takes some time, cancer is primarily a disease of aging. So, how can childhood cancers be explained?

Answer 10.14

In self-renewing tissues the cells contain DNA that has progressively accumulated mutations through multiple DNA replication cycles in progenitor cells (errors in DNA replication and post-replicative DNA repair are frequent causes of mutations).

Pediatric tumors often occur in tissues that are not self-renewing, and such tumors typically have fewer mutations than adult tumors. However, leukemias and lymphomas, which are diseases of self-renewing blood cells, can also often develop early in life. Here the precursor cells are already mobile and invasive and are thought to require fewer DNA changes than in solid tumors, in which the tumor cells require additional mutations to confer these biological capabilities.

Another possibility is that childhood cancers can arise from an initiating mutation that arises in embryonic cells. Progenitor cells in the embryo resemble cancer cells—they are poorly differentiated and rapidly dividing. If they receive a cancer-predisposing mutation, they are much more likely to develop into tumors at an early stage than more differentiated cells with the same mutation.

Question 10.15

List three classes of stromal cell that support the tumor microenvironment.

Answer 10.15

- 1) Infiltrating immune cells (including macrophages, mast cells, neutrophils and T cells)
- 2) Cancer-associated fibroblast cells (including activated tissue fibroblasts)
- 3) Endothelial cells

Question 10.16

There are two fundamental classes of cancer gene in our cells. What are they and what distinguishes them?

Answer 10.16

- 1) Oncogenes are cancer genes that contribute to cancer when the normal allele is present, and so are dominantly-acting cancer susceptibility genes. They are activated by a mutation that results in a change in the gene product (typically a missense mutation that results in a mutant protein) or by a mutation or chromosome rearrangement that either results in activation of expression or in over-expression. Classical oncogenes work in growth signaling pathways to promote cell proliferation or inhibit apoptosis, but as we learn more about the different biological characteristics it is clear that there are also many non-classical oncogenes that also act as dominantly-acting cancer susceptibility genes but that work in some different way other than in a growth signaling pathway.
- 2) Tumor suppressor genes usually contribute to cancer when there is no normal allele, and so are described as recessively-acting cancer susceptibility genes. Typically, both alleles at a tumor suppressor locus must lose their function by an inactivating mutation, or by a chromosomal rearrangement that leads to gene deletion or by epigenetic silencing. Classical tumor suppressor genes work in the opposite direction to classical oncogenes: they suppress cell proliferation or promote apoptosis of deviant cells. But there are many non-classical tumor suppressor genes. For example, some work in maintaining the genome.

Question 10.17

The progression of normal colonic epithelium to colon cancer has been viewed as a multi-stage progression. What is known about how the cancer develops and which are the genes that are most frequently involved?

Answer 10.17

At the phenotype level, the progression can be seen to move from normal colon epithelium to a small benign tumor, an adenoma, and then to a large adenoma and finally to a malignant tumor known as an adenocarcinoma. The process is normally slow, with a benign small adenoma manifesting in patients usually within the age range of 30-50 years, and then another 10 years for the progression to a large adenoma, and a further 10 years to the malignant adenocarcinoma that can invade neighboring tissues.

At the molecular level, the initial driver mutation is almost always one that affects the Wnt signaling pathway, usually the *APC* tumor suppressor gene at 5q21 (which is mutated in about 80% of colorectal tumors). Important downstream contributors include RAS-type oncogenes, notably *KRAS*, and components of the phosphatidylinositide 3-kinase (also known as phosphoinositide 3-kinase) pathway and the transforming growth factor beta pathways. And, as in so many tumors loss of *TP53* alleles is important in accelerating mutation rates.

Question 10.18

Describe three classes of activation mechanism whereby normal cellular oncogenes are activated to become oncogenes.

Answer 10.18

- 1) *Activation by gene amplification.* Tumor cells often contain abnormally large numbers—often hundreds of copies—of a structurally normal oncogene. The *MYCN* oncogene, for example, is frequently amplified in late-stage neuroblasts and in rhabdomyosarcomas; *ERBB2* (also called *HER-2*) is often amplified in breast cancers. The amplification mechanism is not simple tandem amplification; instead, there seem to be complex rearrangements that bring together sequences from several different chromosomes. The amplification may manifest itself in two forms. An extrachromosomal form is known as *double minutes*, which are so called because they are tiny, paired acentric chromatin bodies that are separated from chromosomes and contain multiple copies of just a small set of genes. A corresponding intrachromosomal form (in which multiple repeated copies integrate into chromosomes) gives rise to *homogeneously staining regions*.
- 2) *Translocation-induced gene activation.* Chromosomal translocations occur when DNA molecules receive double-strand breaks and are then re-joined incorrectly so that pieces of different DNA molecules are joined together. When that happens, an oncogene is often inappropriately transcriptionally activated and so there can be a selective growth advantage. Translocations that activate oncogenes are common in cancer. In many cases, the translocations result in the formation of clearly chimeric genes that result in the constitutive expression of oncogene sequences. In other cases, the oncogene sequence is not interrupted by a breakpoint; instead it is simply brought into close proximity to regulatory sequences in another gene that is actively expressed (see Table 10.5 on page 390 for some examples).
- 3) *Gain-of-function mutations.* Oncogenes can be activated by certain point mutations that make a specific change at one of a *few* key codons. Activating mutations in some cellular oncogenes are particularly common, especially when the genes make a product that links different biological pathways connected to cell proliferation and growth. For example, about one in six human cancers has activating mutations in one of the *RAS* genes, most commonly in *KRAS* (which is naturally expressed in almost every tissue). More than 99% of the activating Ras mutations are in one of only three key codons, codon 12 (Gly), codon 13 (Gly), and codon 61 (Gln).

Question 10.19

Cancer is sometimes viewed as a disease of stem cells. What is the evidence?

Answer 10.19

In the cancer stem cell model the cancer cells are maintained by occasional cell division from a limited number of stem cells that are usually sequestered in a safe location (for example, at the very base of intestinal crypts in the case of colon cancer). After an initial asymmetric cell division a stem cell gives rise to two cells: a stem cell like itself and a more differentiated transit amplifying cell. The latter cell can then multiply rapidly through symmetric cell divisions to give large numbers of progeny that eventually go through further differentiation to give rise to post-mitotic differentiated cells.

Supportive evidence for cancer stem cells comes from analyses of many types of leukemia. For example, in chronic myelogenous leukemia (CML) the Philadelphia chromosome (a specific type of chromosomal translocation that involves production of a *BCR-ABL* oncogene) may often be evident in different hematopoietic cell types within CML patients, being found in B and T lymphocytes, neutrophils, granulocytes, megakaryocytes, and so on. The cell in which the Philadelphia chromosome first arose was presumably a precursor of all those different blood cell types, a hematopoietic stem cell.

The idea of cancer stem cells is supported by hierarchical cell organizations for certain types of cancer. In some types of neuroblastoma and myeloid leukemia, for example, the cancer evolves so that some of the tumor cells differentiate and have limited capacity for proliferation, despite retaining the oncogenic mutations of their malignant precursors.

Further evidence comes from flow cytometry, which allows separation of phenotypically distinct sub-populations of live cancer cells. The tumorigenic properties of the subpopulations can then be studied by transplanting them into immunocompromised mice. Using this approach, it became clear that only a small proportion of cancer cells in leukemia and breast cancer proliferate extensively, and they express specific combinations of cell surface markers. For example, breast-cancer-initiating cells were found to express CD44, but showed very little or no expression of CD24; that is, the cancer-initiating cells were found to be CD44⁺CD24⁻ cells, a small minority of the population of tumor cells. Similarly, leukemia-initiating cells were found to be a minority population of CD34⁺CD38⁻ cells. Follow-up studies indicate that many other cancers might also follow the cancer stem cell model.

Explanation 10.19

Definitive proof for cancer stem cells has recently been obtained in the case of myelodysplastic syndromes which have been shown to be propagated by rare and distinct human cancer stem cells in vivo (see Woll PS et al., *Cancer Cell*, in press; PMID 24835589).

Question 10.20

What are double minute chromosomes and homogeneously staining regions, and why do they occur in some cancer cells?

Answer 10.20

Oncogene activation usually occurs by some gain-of-function mutation or by a chromosome rearrangement that somehow causes unexpected activation of gene expression. In some cancer cells, however, an oncogene is activated by gene amplification in which multiple copies are made of a small chromosome region that contains several genes, including a proto-oncogene. Because so many copies of the small chromosomal region gene are made, the expressions of the constituent genes are very strongly increased and the resulting overexpression results in activation of the proto-oncogene to make an oncogene.

The gene amplification may manifest itself in two forms. In one case it is extrachromosomal and results in *double minutes*, which are tiny, paired acentric chromatin bodies that are separated from chromosomes and contain multiple copies of a small set of genes. Sometimes these chromatin bodies integrate into chromosomes and the intrachromosomal forms (in which multiple repeated copies integrate into chromosomes) are described as *homogeneously staining regions*.

Explanation 10.20

See also Figure 10.6B on page 389.

Question 10.21

What is the Philadelphia chromosome and why is it a cause of cancer?

Answer 10.21

The Philadelphia chromosome is a recurring translocation chromosome that is found in 90% of individuals with chronic myeloid leukemia. It results from a balanced reciprocal translocation with breakpoints near the start of the *ABL1* oncogene at 9q34 and close to the end of *BCR* gene at 22q11. The resulting *BCR-ABL1* fusion gene on the Philadelphia chromosome (with the *ABL1* coding sequence positioned downstream of the *BCR* gene sequence and *BCR* promoter) produces a large protein that carries the ABL1 polypeptide sequence at its C-terminal end. This fusion protein acts as a growth-stimulating tyrosine kinase that is *constitutively* active and so drives cell proliferation.

Explanation 10.21

See also Figure 10.7 on page 390.

Question 10.22

The range of different point mutation classes and their locations within coding DNA are major features that differentiate oncogenes from tumor suppressor genes. Explain.

Answer 10.22

Point mutations in tumor suppressor genes are ones that cause loss-of-function, and that can happen in a very large number of different ways. Classical inactivating mutations that cause a premature termination codon to be inserted are common and can be distributed widely across the coding DNA (and also include splice site mutations). But other types of loss-of-function mutations can also be quite frequent, notably missense mutations that cause a non-conservative change to a functionally important amino acid.

The point mutations in oncogenes are different. They need to be gain-of-function mutations and in coding DNA that means missense mutations that lead to a change of amino acid. Unlike loss of function, however, gain of function can happen in only a few precise ways. Substitutions at only certain amino acids are likely to produce a mutant protein that behaves in a quite different way from normal. So, point mutations in oncogenes are dominated by gain-of-function missense mutations and they occur at just a very few codons. For example, in the case of the three human Ras oncogenes that produce highly related proteins with 188 or 189 amino acids, the activating point mutations are confined to gain-of-function mutations that occur at just three codons in each case: codon 12 (Gly), codon 13 (Gly) and codon 61 (Gln).

Explanation 10.22

See also Figure 10.8 on page 392.

Question 10.23

Loci for previously unknown tumor suppressor genes have been mapped to specific chromosomes and specific chromosome regions by screening for loss of heterozygosity. Explain what is involved.

Answer 10.23

Genomic DNA from each of multiple tumors of a specific type is typed with a panel of several hundred highly polymorphic microsatellite DNA markers that are selected to represent marker loci across each human chromosome. The genotypes of some of the markers will show a single allele and that normally would be interpreted to be a homozygous genotype. However, often a tumor suppressor allele is eliminated by loss of a whole chromosome or sometimes by a chromosome rearrangement that leads to loss of the chromosome segment containing the tumor suppressor loci. In such cases, all the markers from the same chromosome or chromosome region will appear to be homozygous, but are in fact *hemizygous*. The chances that multiple neighboring highly polymorphic marker loci are homozygous would be negligible and instead strongly indicate that there is a tumor suppressor locus on the chromosome or subchromosomal region that shows this phenomenon. If the same finding is recorded in several tumors of the same type the evidence is overwhelming.

Question 10.24

Tumor suppressor genes have been classified into caretaker, gatekeeper and landscaper categories. What is meant by these categories? Illustrate your answer with examples for the first two categories.

Answer 10.24

- Caretaker genes have roles in genome maintenance, notably in DNA repair. If both alleles are inactivated, the mutation rate accelerates which can then lead to increased chances of mutation in other cancer susceptibility genes. Examples include the important breast cancer susceptibility genes *BRCA1* and *BRCA2* that work normally in cells as components of the homologous recombination-based double-strand DNA repair pathway, and genes involved in mismatch repair, such as *MLH1* and *MSH2*.
- Gatekeeper genes are the classical tumor suppressor genes that work to promote net cell proliferation. Some of them directly regulate the cell cycle/cell growth (by acting as brakes on cell growth cycle, suppressing the G1-S transition and inducing cell cycle arrest, as required, or by working in upstream growth signaling pathways. Others promote apoptosis. Examples include the *RBI* gene, which is mutated in retinoblastoma.
- Landscaper genes are important in regulating the stromal environment.

Question 10.25

Not all cancer-susceptibility genes make proteins – some make noncoding RNAs. Give three examples of cancer-susceptibility genes that make long noncoding RNAs and explain how they are involved in cancer.

Answer 10.25

Any three from the list below.

ANRIL	Represses expression of both p14 and p16 tumor suppressors; upregulated in prostate cancer
H19	Ectopic expression promotes cell proliferation; upregulated in gastric cancer
HOTAIR	Promotes cancer metastasis; upregulated in breast, gastric and colorectal cancers
PTENP1	Regulator of the PTEN tumor suppressor gene; lost in many human cancers
XIST	Involved in X-chromosome inactivation but down-regulated in various cancer cell lines and a powerful suppressor of hematological malignancies in vivo in mice

Question 10.26

Not all cancer-susceptibility genes make proteins – some make noncoding RNAs. Give two examples of miRNA genes that act as cancer-susceptibility genes and explain how they are involved in cancer.

Answer 10.26

- 1) The *MIR15A* and *MIR16-1* genes normally induce apoptosis by targeting BCL-2, and are frequently deleted or down regulated in chronic lymphocytic leukemia.
- 2) *MIR21* stimulates proliferation and tumor initiation by repressing pro-apoptotic genes such as *PTEN* and is strongly upregulated in many types of cancer.

Question 10.27

Illustrate how some tumor suppressor genes are non-classical in the sense that they can make a significant contribution to tumorigenesis after losing just one allele.

Answer 10.27

Classical tumor suppressor genes conform to the two-hit hypothesis: they contribute to tumorigenesis after both alleles have been inactivated by gene deletion, mutational inactivation or epigenetic silencing. However, some non-classical tumor suppressor genes contribute to tumorigenesis even after one allele is lost or inactivated (haploinsufficiency). The effect of loss of function of one allele is not as great as when both alleles are lost/inactivated, but can nevertheless be important. For example, loss of just a single *BRCA1* allele has been shown to lead to genome instability in cultured cells (and in animal models).

Just like haploinsufficiency in single gene disorders, haploinsufficiency for tumor suppressor genes is a feature of dosage-sensitive genes. The dosage effects can be tissue-specific and dependent on the genetic context, such as the genetic background. The *PTEN* tumor suppressor gene provides clear evidence of dosage effects that also show some tissue-specificity. For example loss of both *PTEN* alleles in prostate tissue results either in cell senescence (if there is a wild type p53 in the cell) or an aggressive cancer (if there is a mutant p53), but when there is 50% of the normal levels of *PTEN* the result is a milder phenotype (prostatic intraepithelial neoplasia, which is effectively a precursor of a prostatic carcinoma). In the case of blood cells, loss of both *PTEN* alleles in an otherwise normal genetic background leads to hematopoietic stem cell exhaustion and bone marrow failure or leukemia (when aneuploidy, additional cancer mutations and mutant p53 are present). But when there is 50% of the normal levels of *PTEN* in blood cells the result is lymphadenopathy, splenomegaly and lymphoma.

Explanation 10.27

See Figure 10.13 on page 398 for further details.

Question 10.28

Illustrate how some tumor suppressor genes are non-classical in the sense that they preferentially acquire missense mutations.

Answer 10.28

The most celebrated example of a tumor suppressor gene in which missense mutations are common is the *TP53* gene. Unlike classical tumor suppressors where inactivating mutations are very common, the great majority of small-scale cancer-associated mutations in *TP53* are missense mutations that are very largely clustered within the central DNA-binding domain. The mutated p53 proteins have multiple properties that distinguish them from wild-type p53. First, unlike wild-type p53, mutant p53s do not participate in a self-limiting regulation. In normal cells, the amount of p53 is kept low because p53 is negatively regulated by MDM2 (and MDM4), and p53 positively regulates the production of its major antagonist MDM2; in cells with missense mutations in *TP53*, large amounts of mutant p53 are produced because mutant p53 fails to stimulate the production of MDM2.

Mutant p53 proteins also work in a dominant-negative fashion. A missense mutation in the *TP53* gene can result in much greater loss of p53 function than a null mutation because the normal working form of p53 is a tetramer. This happens in much the same way as shown for dominant negative mutations in collagen disorders*, but because p53 is a tetramer, a mutant subunit has a chance of being included in 15 out of 16 tetramers and so whereas a null mutation in one allele leads to about 50% loss of p53 function, a missense mutation that produces a dominant negative p53 protein leads to $15/16 = 94\%$ loss of p53 function, (making a second hit in the two-hit tumor suppressor process much more likely).

Mutant p53 also suppresses the related p63 and p73 transcription factors (which show high sequence homology to p53 in some domains), and it antagonizes the interaction of wild-type p53 and the recognition sequences it must bind in its target genes. In addition, the mutant p53 works as a rather different type of transcription factor by stimulating transcription of quite different target genes, including many genes that stimulate cellular proliferation or that inhibit apoptosis.

Explanation 10.28

See also Box 10.13, Figures 2 and 3 on pages 400-401. *For dominant negative mutations in collagen disorders, see pages 226-227 and Figure 7.17.

Question 10.29

How are MSI-positive tumors recognized and what does the MSI-positive property signify?

Answer 10.29

MSI-positive tumors mean tumors that show evidence of microsatellite instability. That can be identified when genomic DNA from tumors is typed for a variety of highly polymorphic microsatellite DNA markers. Normally, two major bands or peaks would be seen for

heterozygous genotypes but some tumors will show patterns that are more complicated, with extra major bands, indicating that the alleles at the microsatellite loci are unstable. Microsatellite instability reflects defective mismatch repair, which in turn is due to mutations in key genes that work in mismatch repair. The result of defective mismatch repair is that the mutation rate across the genome accelerates, and leads to a much higher chance of other cancer-susceptibility genes being mutated.

Question 10.30

Defective mismatch repair can occasionally occur in some other types of tumor, but it is particularly common in colorectal cancer. Why should that be?

Answer 10.30

One explanation is that MMR deficiency sabotages a key defense system that protects against colorectal cell proliferation. In the colorectum, transforming growth factor β (TGF β) is a particularly strong inhibitor of cell proliferation, and it specifically binds to a receptor on the surface of the cells of which the TGFBR2 protein is a key component. However, the *TGFBR2* gene is readily inactivated as a result of mismatch repair deficiency because it has a long sequence of adenines that is liable to replication slippage, causing frameshifting insertions and deletions. Somatic mutations in *TGFBR2* are found in about 30% of sporadic colorectal cancer but are very frequent in colorectal cancer that shows evidence of microsatellite instability.

Explanation 10.30

Figure 10.18 on page 406 shows the long sequence of adenines in the coding sequence of the *TGFBR2* gene.

Question 10.31

Match cancers a) to d) with one of the descriptions i) to iv)

Cancer

- a) adenocarcinoma
- b) papilloma
- c) squamous cell carcinoma
- d) adenoma

Description

- i) a benign tumor of epithelial tissue
- ii) a malignant tumor of multilayer epithelial tissue
- iii) a malignant tumor of epithelial origin
- iv) a benign tumor of multilayer epithelial tissue

Answer 10.31

- a) iii)

- b) iv)
- c) ii)
- d) i)

Question 10.32

The number of mutations in a cancer cell can vary. Match the different types of tumors listed in a) to e) with one of the three ranges for numbers of somatic substitutions per tumor given in i) to iii).

Type of tumor

Somatic substitution range

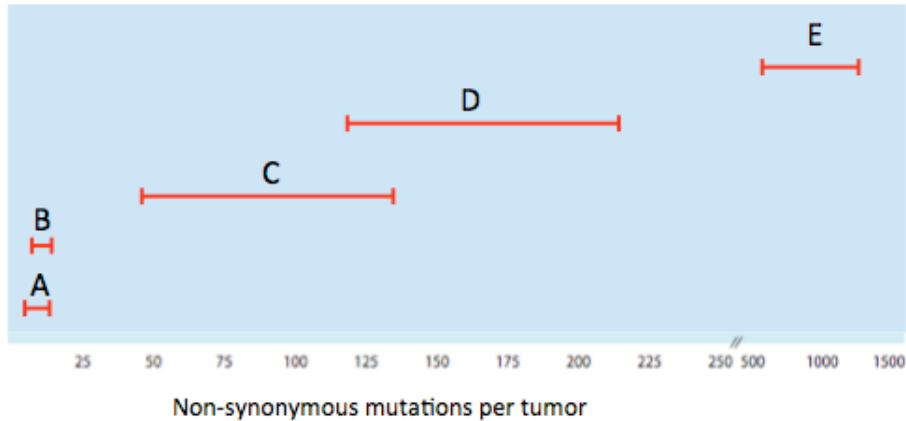
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|-----------------------|--|
| a) lung cancer | i) usually less than 1000 |
| b) most adult cancers | ii) 1000-10,000 |
| c) pediatric tumors | iii) significantly greater than 10,000 |
| d) melanoma | |
| e) liquid tumors | |

Answer 10.32

- a) iii)
- b) ii)
- c) i)
- d) iii)
- e) i)

Question 10.33

In the figure below, which of the following are A to E likely to represent?
 chronic lymphocyte leukemia; melanoma; medulloblastoma; microsatellite instability (MSI)-positive colorectal cancer; MSI-negative colorectal cancer.



Answer 10.33

- A) medulloblastoma (pediatric tumor)
- B) chronic lymphocyte leukemia (liquid tumor)
- C) MSI-negative colorectal cancer (adult solid cancer)
- D) melanoma (mutagen-induced)
- E) MSI-positive colorectal cancer

Question 10.34

Recent studies have shown that non-classical cancer genes link metabolism to the epigenome. Explain the connection.

Answer 10.34

The *IDH1* and *IDH2* genes make cytosolic and mitochondrial isocitrate dehydrogenase, enzymes that work in the tricarboxylic acid (Krebs) cycle to convert isocitrate to 2-oxoglutarate also known as α -ketoglutarate). One of these two genes is (heterozygously) mutated in 80–90% of adult grade II/III gliomas and secondary glioblastoma, in more than 50% of chondrosarcomas, in a significant proportion of acute myeloid leukemias, and in some other cancers.

The predominant *IDH1/IDH2* cancer-associated mutations are specific missense mutations producing mutant enzymes that convert 2-oxo-glutarate (produced by the normal allele) to 2-hydroxyglutarate. At high concentrations, 2-hydroxyglutarate inhibits multiple enzymes that depend on 2-oxoglutarate as a cofactor and that work in epigenetic modification. The inhibited enzymes include certain DNA demethylases, such as TET2, and various histone demethylases; epigenetic reprogramming of the cell results, making it less differentiated.

As well as oncogenes, tumor suppressor genes regulate the epigenetic– metabolic link in cancer cells. For example of the *SIRT6* tumor suppressor gene encodes a histone deacetylase that normally suppresses aerobic glycolysis.

Question 10.35

The biological hallmarks of cancer are regulated by partially redundant signalling pathways and that can pose very significant challenges to therapeutic approaches that seek to inhibit a specific biological hallmark of cancer. Explain the challenges posed when using inhibitors of telomerase and of angiogenesis.

Answer 10.35

The problem here is that using one approach to inhibit a specific biological hallmark of cancer – be it cancer cell immortality or the dependence of cancer cells on angiogenesis – may not shut down the relevant biological capability: an alternative pathway can be activated to ensure tumor survival.

Telomerase inhibitors. Cancer development is strongly assisted by switching on telomerase; but in response to drugs that inhibit telomerase, it is common for cancer cells to find alternative ways of maintaining telomeres to ensure continued cell proliferation. A telomerase-independent alternative mechanism known as ALT (alternative lengthening of telomeres) is naturally used by the minority of human tumor cells that lack significant telomerase. It appears to involve sequence exchanges between different telomeres and is not an easy therapeutic target.

Angiogenesis inhibitors. During the development of the vasculature in normal embryogenesis, new endothelial cells are formed and assembled into tubes (vasculogenesis); in addition, new blood vessels can sprout from existing ones (angiogenesis). Thereafter, the normal vasculature becomes largely quiescent. But during processes such as wound healing, angiogenesis can be turned on in adults, if only transiently. Like normal tissues, tumors need nutrients and oxygen and must get rid of metabolic waste and carbon dioxide. To fulfill these needs, angiogenesis is almost always activated and remains switched on to generate new tumor-associated blood vessels.

In some preclinical models, potent inhibitors of angiogenesis are successful in suppressing this property; however, when confronted with angiogenesis inhibitors, tumors simply adapt by developing enhanced properties of tissue invasion and metastasis. By invading nearby tissues, cancer cells can gain access to alternative sources of preexisting tissue vasculature.

Question 10.36

What is meant by targeted cancer therapy? Illustrate your answer with reference to treatment for chronic myeloid leukemia.

Answer 10.36

Targeted cancer therapy becomes possible after genetic studies have defined a precise molecular focus for therapy. Thus, for many cancers, genetic analyses have identified an aberrant gene product that results from the mutation of a driver gene and which is highly characteristic of that cancer. In that case, conventional small molecule drugs or specific monoclonal antibodies might be directed to treating the cancer with considerable success.

The paradigm of targeted cancer therapy has been the treatment of chronic myeloid leukemia (CML) with imatinib. CML accounts for 20% of adult leukemia, and more than 90% of CML cases have the Philadelphia translocation chromosome (with breakpoints in the *ABL1* proto-oncogene and *BCR* genes). The resulting hybrid gene makes the BCR–ABL1 fusion protein that is a constitutively active tyrosine kinase powered by ATP. The small molecule drug imatinib (marketed as Gleevec™) was developed to inhibit this tyrosine kinase by acting as a competitive inhibitor—it binds to the ATP binding site of the kinase. By blocking ATP binding, imatinib prevents the switch from the inactive enzyme conformation to the active conformation. The specificity of imatinib is not perfect (it inhibits certain other kinases in addition to ABL1 kinase). It has nevertheless transformed the treatment of CML: it became the first-line therapy for CML almost immediately after its introduction, and has had very significant success in prolonging survival times. Because resistance to imatinib can be conferred by point mutations within the ABL1 kinase domain, second-generation tyrosine kinase inhibitors (notably nilotinib and dasatinib) are used to provide alternative treatments.

Question 10.37

Tumor recurrence is a major problem in cancer gene therapy. Why should tumors recur so readily?

Answer 10.37

Cancer stem cells. One possibility is that cancer stem cells might be relatively resistant to therapy, thus surviving to repopulate a vastly shrunken tumor. This idea has received experimental support. For example, in a genetically engineered mouse model of glioblastoma, a relatively quiescent subset of endogenous glioma cells (with properties resembling cancer stem cells) was recently found to be responsible for sustaining long-term tumor growth through the production of transient populations of highly proliferative cells. If cancer stem cells are relatively resistant to therapy, there would be the problem of how to effectively target and kill populations of cancer stem cells about which we know little.

Tumor heterogeneity. If a malignant tumor consists of genetically different populations, some cells might survive drug treatment and natural selection could foster the development of tumor subclones with mutations that render the therapeutic drug ineffective in some way. (There are parallels here with infectious diseases and the evolution of drug resistance in microbes.)

Question 10.38

Give three examples of mechanisms that explain the evolution of drug resistance in tumors.

Answer 10.38

1. *Mutations in the gene encoding the drug target.* For example, in the treatment of chronic myeloid leukemia with imatinib, tumor subclones develop imatinib resistance by

developing point mutations that alter the kinase domain of the BCR– ABL1 protein. The mutant kinase retains the catalytic activity required for tumor formation, but imatinib can no longer bind to it effectively to inhibit it. Drug resistance for many other kinase inhibitors works by a similar mechanism: often the mutations confer resistance by blocking interactions between drug and target through steric hindrance.

2. *Amplification of the gene encoding the drug target.* Occasionally, for example, resistance to kinase inhibitors in CML is achieved when tumors succeed in amplifying the *BCR–ABL1* gene. Prostate cancers often acquire resistance to drug-mediated androgen deprivation by amplifying the androgen receptor gene.
3. *Bypassing the primary drug target* (which remains unaltered, and continues to be inhibited by the drug). This can take the form of mutating a downstream effector in the same pathway to render cells insensitive to drug inhibition of a cell surface receptor, for example; or an alternative pathway is activated. For example, the monoclonal antibody trastuzumab is designed to treat breast cancer by binding to and interfering with the human epidermal growth factor receptor 2 (HER2), but tumors can bypass the effects of the drug by activating expression of the alternative receptors, including HER3.