Questions and Answers for Genetics and Genomics in Medicine Chapter 9

Question 1

Treatment of genetic disorders sometimes involves augmentation therapies. What does this mean? Give examples of how augmentation therapy can be deployed at the following levels: (a) somatic phenotype; (b) cellular level; (c) gene level; (d) gene product/metabolite level.

Answer

Augmentation therapies seek to treat genetic disorders in which the root cause is a deficiency. It may be a deficiency that is treated at the phenotype level, such as loss of vision or loss of hearing, or at the cellular level by transplanting cells that the patient lacks, or at the molecular level to provide a functioning gene, gene product, or downstream substance that the patient lacks. Examples of augmentation therapy include the following:

(a) providing cochlear implants or hearing aids to treat hereditary deafness

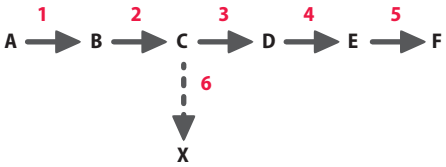
(b) bone marrow transplantation to treat various disorders where certain types of blood cell are depleted; gene therapies using genetically modified cells

(c) injection of cDNA constructs into muscle cells to express a missing protein

(d) treatment of hemophilia with the purified clotting factor; treatment of diabetes with insulin; treatment of congenital hypothyroidism with purified thyroid hormone.

Question 2

In the metabolic pathway below, a major pathway that converts metabolite A to metabolite F in five steps is controlled by enzymes 1 to 5. In addition, trace amounts of metabolite X are normally produced in a minor side pathway.



If the gene that produces enzyme 4 were to be homozygously inactivated, how would you expect the concentrations of the different metabolites to be affected? The genetic deficiency in enzyme 4 might produce a disease phenotype that could be treated by augmentation therapy, and it could simultaneously result in a disease phenotype that is not treatable by augmentation therapy. Explain how the two different disease phenotypes could arise.

Answer

Deficiency in enzyme 4 will mean that the concentrations of E and F will fall markedly and there will be a buildup of substrate D. Increased concentrations of D will drive the equilibrium of the reaction catalyzed by enzyme 3 toward making much more of the upstream products, notably product C, and increased concentrations of metabolite C can drive a greatly increased production of metabolite X.

The disease consequences of the altered changes in metabolite concentration depend on the extent to which failure to make enzyme 4 is catastrophic or not. If there is no other enzyme that can partly substitute for its function, it might lead to a recessive disorder and the loss of function would be treatable, in principle, by augmentation therapy.

Metabolite X might normally be produced in tiny quantities because in high concentrations it could be toxic to cells. Genetic deficiency in enzyme 4 (or enzyme 3) would then result in abnormally high concentrations of X, and an additional disease phenotype could arise because of the resulting toxicity that would not be treatable by augmentation therapy.

Question 3

Genes that encode drug-handling enzymes often show quite high levels of polymorphism. Why should that be?

Answer

Genetic variation is most highly developed in genes that work in recognizing foreign molecules introduced into the body that are under independent genetic control. Components of invading microorganisms are specifically detected by antibodies and T-cell receptors that are designed to be genetically diverse to provide extra protection as these organisms mutate (in an effort to avoid being recognized by our immune systems). Because we are also at risk of consuming toxic molecules that are under genetic control (such as plant and fungal toxins), many enzymes that are involved in dietary metabolism are also polymorphic. Because they also handle the metabolism of artificial drugs, genetic variation in these enzymes explains the wide variation between individuals in the ways in which we metabolize and respond to drugs.

Question 4

For some drugs there is a narrow therapeutic window. With reference to the anticoagulant warfarin, explain what this means.

Answer

The therapeutic window for a drug is the range of concentrations within which the therapeutic benefit is optimal without posing any great risks to health. If the concentration is below this range, the therapeutic benefit might be insufficient (drug underdose); if above this range, there is an increasing risk of toxicity (drug overdose).

Warfarin is prescribed for patients at risk of developing clots within blood vessels (thrombosis), including clotting that can block arteries (embolism). Delivering the optimal warfarin dosage is clinically very important because there is a narrow therapeutic window: if the administered warfarin level is too low, the patient remains at risk of thrombosis and embolism; if it is too high, there is a risk of life-threatening hemorrhage. The final warfarin dose is critical, but because of genetic variation the optimal dose varies enormously between individuals.

Question 5

Genetically engineered antibodies have become an important class of therapeutic drug. Describe briefly the different classes of genetically engineered antibody and their applicability to treating genetic disorders.

Answer

There are two fundamental classes of genetically engineered antibodies. One has the conventional antibody structure with two heavy chains and two light chains, each containing variable and constant domains and being held together by disulphide bonds. The second class has a single chain with just two variable domains that are held together by a linker polypeptide sequence, a so-called scFV (single chain, variable fragment) antibody. In each case the genetically engineered antibody is designed to bind specifically to some harmful protein (or other molecule) that causes the pathogenesis, in an effort to block how the harmful protein works.

A conventional monoclonal antibody produced in a rodent and injected into a patient has limited therapeutic potential: it typically has a short half-life in human serum, and the human patient often produces anti-rodent antibodies. Genetically engineered antibodies with the conventional antibody structure are designed to be more stable in human serum and less likely to elicit an immune response. That is achieved by swapping human sequences for the original rodent sequences at the gene level; the resulting genetic constructs make hybrid (part human and part rodent) antibodies or fully human antibodies. A chimeric V/C antibody is one in which all the constant domains are swapped so that the only parts of the antibody that remain rodent are the variable domains. A humanized antibody takes it one level further: here, all of the antibody is of human origin, except for the complementarity-determining regions of the variable domains. A full swap produces a fully human antibody. Examples of all three of these subclasses have been used in disease treatment.

The scFV antibodies have almost all the binding specificity of a conventional monoclonal antibody but are restricted to a single non-glycosylated variable chain. They can be made on a large scale in bacterial, yeast, or even plant cells. Because they are stable in the reducing environment within cells, they are well suited to acting as intracellular antibodies (intrabodies) and can be designed to bind specific target molecules within cells; they can also be directed as required to specific subcellular compartments. Although they are seen to have great therapeutic potential, this development is largely still at the research stage.

Question 6

What are the main advantages and disadvantages of using retrovirus and adenovirus vectors in gene therapy?

Answer

Retrovirus vector advantage:

- An integrating vector. It allows therapeutic genes to be packaged into chromosomes so that they can be stably transmitted to daughter cells, allowing the possibility of long-lasting therapeutic benefit if the therapeutic genes integrate into the chromosomal DNA of stem cells.

Retrovirus vector disadvantages:

- Comparatively modest levels of gene expression.

- A safety profile that can be a concern. Integration into chromosomal DNA occurs in a semi-random way, and in various clinical trials for blood disorders it has led to the unexpected activation of oncogenes, causing leukemia.

Adenovirus vector advantage:
- High-level expression of therapeutic transgenes.

Adenovirus vector disadvantages:
- A non-integrating vector and so not so suitable for short-lived target cells, such as blood cells.
- A safety profile that can be a concern. The therapy requires the repeated administration of high titers of recombinant adenovirus, which can induce adverse immune reactions that can be fatal.

Question 7

Genetically modified mice are frequently used to model human disorders by introducing mutations into the mouse germ line. This is most often achieved by two methods that involve either gene targeting of mouse embryonic stem cells or inserting a transgene into the zygote. What classes of human genetic disorder are most readily modeled by each of these methods?

Answer

Gene targeting via mouse embryonic stem cells. The most frequent human disorders to be modeled in this way are recessive conditions. We know that any homozygous mutation that inactivates the equivalent mouse gene is likely to have a similar effect on the phenotype. Less readily, other types of pathogenic mutation, such as individual missense mutations, can be replicated in mice in an effort to model the corresponding human phenotype.

Transgene injection into the mouse zygote. This method is well suited to modeling human phenotypes that arise from gain-of-function mutations. Sometimes a mutant mouse transgene is used in which the equivalent mouse cDNA is engineered to have the same type of mutation as the human gain-of-function mutation, or in some cases a human mutant transgene is used.