**Diagnostic Molecular Pathology – Case Studies and Questions**

**Chapter 1**

***Basic Concepts in Molecular Pathology – Introduction to Molecular Testing in Human Disease***

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**Case 1 – Genetics and Epigenetics of Disease**

Contemporary descriptions of diseases and their associated molecular mechanisms often refer to genetic or genomic changes, as well as epigenetic changes.

***Which of the following statements are true regarding molecular mechanisms of disease?***

1. Genomic alterations include point mutations, small insertions, small deletions, and chromosomal abnormalities.
2. Copy number changes are considered genomic alterations.
3. Epigenetic changes include any alteration in the genome that does not involve changes in the primary sequence of the DNA.
4. Epigenetic alterations include aberrant DNA hypermethylation or hypomethylation, and/or abnormal histone modifications resulting in alterations in chromatin structure.
5. All of the above.
6. None of the above.

**Correct Answer:** E

**Explanation:** Genomic changes include any alterations affecting the sequence of DNA. This includes small sequence alterations (point mutations, deletions, insertions), large sequence alterations (large-scale deletions or insertions), and chromosomal alterations (numerical or structural). Epigenetic alterations include changes to the genome that do not involve changes to the DNA sequence, including (but not limited to) changes in DNA methylation and histone modifications.

***Which of the following represent consequences of genetic alterations (mutation) and/or epigenetic alterations (epimutation)?***

1. Loss or reduction of normal levels of gene expression with consequent loss of protein function
2. Loss of function due to loss of protein or synthesis of defective protein
3. Increased levels of gene expression with consequent overexpression of protein
4. Gain-of-function mutation with consequent altered protein function.
5. All of the above
6. None of the above

**Correct Answer:** E

Explanation: Both epigenetic (like DNA hypermethylation) and genetic alterations (gene deletion for instance) may lead to loss of gene expression, which leads to reduced levels of protein expression (and loss of function). Loss of protein function can be the result of loss of protein expression or synthesis of a defective protein in the case of mutation (change of amino acid in an active site or change of protein folding). Both epigenetic (like DNA hypomethylation) and genetic alterations (chromosomal translocation for instance) may lead to increased levels of gene expression and consequent protein overexpression. An excellent example of a gain-of-function mutation is the activating mutation of K-ras in cancer cells, where a single amino acid change results in a constitutively active protein. This is a genetic change (with no epigenetic equivalent).

**Case 2: Sources of Nucleic Acids for Molecular Testing**

To support molecular testing in the workup of patients with known or suspected disease requires an adequate source of nucleic acids for use as biomarkers in molecular diagnostic assays. There are numerous potential sources for patient-derived DNA. These sources can be divided based upon the difficulty in sampling and/or the discomfort to the patient during sampling as (i) invasive sources of DNA biomarkers, or (ii) non-invasive sources of DNA biomarkers.

***Which of the following accurately describe non-invasive sources of DNA biomarkers?***

1. Urine is a non-invasive source of DNA biomarkers
2. Non-invasive sources of DNA cause minimal to no discomfort to the patient
3. Blood samples can be collected with minimal discomfort to the patient and so are considered non-invasive
4. Spinal fluid is a non-invasive source of DNA biomarkers
5. All of the above
6. A, B, and C, but not D

**Correct Answer:** F

**Explanation:** Non-invasive sources of DNA can be collected from the patient with minimal to no discomfort. Urine is an excellent example of a source for DNA biomarkers that causes no discomfort to patients upon collection, while blood collection is associated with minimum discomfort. Hence, urine and blood are considered non-invasive sources of DNA biomarkers. In contrast, spinal fluid is collected through an invasive spinal tap procedure that is associated with patient discomfort and so is not considered non-invasive.

***Which of the following accurately describe invasive sources of DNA biomarkers?***

1. Tissue biopsies are collected through an invasive procedure, but can provide DNA biomarkers for testing
2. Surgical specimens are considered non-invasive and can provide DNA biomarkers for testing
3. Urine is collected through an invasive procedure
4. Pap smear specimens represent excellent sources of DNA biomarkers collected through a non-invasive procedure
5. All of the above
6. None of the above

**Correct Answer:** A

**Explanation:** Tissue biopsies, surgical specimens and Pap smear specimens all represent excellent sources of DNA biomarkers for testing, but each is collected through an invasive procedure. Any surgical procedure or procedure that causes pain and discomfort to the patient is considered invasive. Urine is the prototype for a non-invasive source of DNA biomarkers since it can be collected with no discomfort to patients.

***What is the desired product from invasive or non-invasive sample collection?***

1. Cells corresponding to diseased tissue
2. Cell-free DNA
3. Metabolites contained in the fluid
4. A and B
5. B and C
6. All of the above

**Correct Answer:** D

**Explanation:** Most molecular assays utilize DNA biomarkers. Hence, DNA from diseased tissue or cells is the desired product of these sampling procedures (whether invasive or non-invasive). Cells from diseased tissues can yield DNA for testing, and in some cases cell-free DNA (originating from diseased cells and tissues) is isolated.

**Case 3 – Molecular Classification of Disease**

Evidence for the existence of molecular subtypes of disease includes the observation by clinicians that patients with the same disease diagnosis and diseases that share many phenotypic characteristics will display widely varying clinical courses and responses to therapy. Hence, when a cohort of patients with a given disease are treated with a common standard therapy, only a subset of patients will respond favorably. This suggests that the descriptive classification of diseases is inadequate to predict clinical outcomes with accuracy.

***Which of the following statements regarding the molecular classification of disease are true?***

1. Molecular classification of disease can be accomplished using gene expression profiles
2. Molecular classification of disease can be accomplished using genomic characteristics (aberrant chromosomes and copy number variations)
3. Molecular classification is a more reliable predictor of outcomes than descriptive classifications
4. All of the above
5. None of the above

**Correct Answer:** D

**Explanation:** Early methods for molecular classification of disease involved gene expression patterns from microarray datasets. More recently, molecular classification has been accomplished using smaller gene expression datasets (based upon real-time PCR or next generation sequencing). More recent studies have shown that other genomic characteristics can be useful in molecular classification of disease. No matter the methodology used for molecular classification of disease, in many cases the molecular subtypes are superior to descriptive classifications with respect to prediction of various clinical outcomes.

**Case 4 – Molecular Detection of Disease**

Molecular biomarkers are frequently utilized for classification of disease and/or identification of critical molecular features of disease, but also have value in the detection of occult (sub-clinical) disease. The idea of population-based screening of people for detection of disease at a very early stage is attractive, especially if it can be accomplished using non-invasive sources of biomarkers.

***Which of the following enable molecular detection of disease?***

1. Availability of sensitive methods (such as PCR) for detection of low quantities of biomarkers
2. Low costs associated with laboratory methods (such as PCR) used in molecular detection of disease
3. Availability of archived pathology specimens
4. A and B
5. B and C
6. All of the above

**Correct Answer:** D

**Explanation:** The ability of detect disease at a very early point in its natural history requires sensitive methods for detection of biomarkers. PCR is the prototypical laboratory method for rapid and cost effective detection of biomarkers of low abundance. Practical application of the molecular detection of disease will be accomplished using various sources of biomarkers (preferably non-invasive sources like blood, sputum, or urine), but not archived specimens (like paraffin-embedded tissues).

***Which of the following represent limitations to the practical application of molecular detection of disease?***

1. Generation of false-positive results using the molecular assay
2. Generation of false-negative results using the molecular assay
3. Generation of true-positive results in patients that will not develop clinical disease
4. Generation of true-negative results in patients that develop clinical disease
5. All of these
6. None of these

**Correct Answer:** E

**Explanation:** Sensitivity and specificity are important performance parameters for any molecular test. Generation of excess false-positive or false-negative results using the molecular assay will diminish its usefulness in the detection of disease. Equally important to the overall performance of the molecular assay is the generation of true-positive results where the molecular defect associated with disease is correctly detected. However, it is known that molecular lesions can be detected in some patients that will not develop clinical disease due to failure of the abnormal cells to persist in the patient. Thus, extreme caution is required when interpreting molecular tests. Further, in some cases, molecular lesions may not be detected (true-negative result) but the patient develops disease nonetheless. This could be attributed to alternative molecular targets and pathways being activated leading to disease, or the presence of abnormal cells at levels below the sensitivity of the assay.

**Case 5 – Molecular Detection of Lung Cancer**

Early detection of lung cancer is essential to the successful treatment of the disease and long-term patient survival. Lung cancer also tends to occur among smokers and people with other identifiable risk factors. Hence, molecular testing for lung cancer is a practical idea in that people at-risk are easily identifiable for screening, and early detection will greatly benefit the patient. Occult lung cancer has been detected through molecular testing for gene mutations in sputum.

***Which of the following statements are true related to molecular detection of occult lung cancer?***

1. Occult lung cancer can be detected in sputum secondary to identification of mutations in driver oncogenes (like K-ras)
2. Occult lung cancer can be detected in sputum secondary to identification of mutations in recessive tumor suppressor genes (like p53)
3. Sputum samples that are positive for gene mutations are also cytologically-positive enabling pathologic confirmation of diagnosis
4. Cytological diagnosis of lung cancer in sputum requires confirmation by molecular testing
5. A and B
6. C and D
7. None of these

**Correct Answer:** E

**Explanation:** Occult lung cancer can be detected in cytologically-negative sputum samples through identification of mutant K-ras or p53 genes. Hence, sputum samples may be cytologically-negative, preventing a pathological diagnosis of lung cancer. When sputum cytology reveals lung cancer, no molecular confirmation is required.

***Which of the following statements are true related to molecular detection of occult lung cancer secondary to identification of K-ras or p53 mutations in at-risk individuals?***

1. Failure to identify a K-ras or p53 mutation indicates no risk of lung cancer
2. Identification of a K-ras or p53 mutation indicates lung cancer has developed
3. K-ras or p53 mutations in sputum identifies an individual that will progress to clinical lung cancer
4. Not all people with a K-ras or p53 mutation in their sputum will develop clinical lung cancer
5. All at-risk individuals with a K-ras or p53 mutation should be treated with chemotherapy

**Correct Answer:** D

**Explanation:** Whereas occult lung cancer can be detected in cytologically-negative sputum samples through identification of mutant K-ras or p53 genes, studies have shown that not all individuals with a detectable mutation will progress to clinical disease. This observation reflects the fact that molecular testing can identify very early precursors of lung cancer and not all of these aberrant cells will survive and progress. Hence, detection of these molecular alterations in the absence of cytological (or other) evidence of disease increases one’s risk, but does not equate to a diagnosis of lung cancer which requires treatment.

**Case 6 – Molecular Diagnosis and Prognosis of Disease**

Results from molecular testing can provide insights into the intrinsic properties of disease states that are commonly diagnosed using broad classifications based upon tissue of origin and histopathologic features of the lesion. Breast cancer is one such disease. It is recognized to be heterogeneous and clinical classification is based upon expression or lack of expression of several immunohistochemical markers (ER, PR, and HER2). Triple-negative breast cancers do not express ER, PR, or HER2.

***Which of the following statements are true related to molecular subtyping of triple-negative breast cancer?***

1. Molecular subtypes among triple-negative breast cancers are identified using immunohistochemical markers
2. Molecular subtypes among triple-negative breast cancers are identified based upon gene expression patterns
3. Different molecular subtypes of triple-negative breast cancer are differentially sensitive (or responsive) to specific chemotherapeutic drugs
4. A and B
5. B and C
6. A, B, and C

**Correct Answer:** E

**Explanation:** Triple-negative is a clinical classification of breast cancer that is based upon the absence of traditional breast cancer biomarkers (ER, PR, and HER2) by immunostaining. Molecular subtypes of triple-negative breast cancer are revealed through analysis of gene expression patterns, and these molecular subtypes have been associated with differential sensitivity to chemotherapeutic drugs. Hence, knowing the molecular subtype of a triple-negative breast cancer may benefit treatment decisions for the individual patient.

***Which of the following statements is false regarding molecular subtyping of breast cancer?***

1. Gene expression patterns represent the basis for molecular subtyping of breast cancer
2. Other molecular features (such as gene copy number and gene mutations) can be used as a basis for molecular classification of breast cancer
3. Several different sets of genes are equally effective at molecular subtyping of breast cancer
4. A and B
5. B and C

**Correct Answer:** A

**Explanation:** Gene expression patterns represent the original basis for molecular classification of breast cancer. However, more recent classification schemes often take into account gene copy numbers, chromosomal aberrations, absence or presence of gene mutations, and other features. The original intrinsic subtypes of breast cancer were identified by different research groups using different (non-overlapping) sets of genes.

***Which of the following statements is false regarding the use of molecular subtyping to predict breast cancer patient outcomes?***

1. Gene expression patterns associated with selected genes in specific molecular assays can predict outcomes among breast cancer patients
2. Prognostication of breast cancer can be accomplished based upon molecular subtype alone
3. Prediction of patient outcomes in breast cancer can be accomplished using the gene expression patterns of a relatively small number of genes
4. A and B
5. B and C

**Correct Answer:** B

**Explanation:** Gene expression patterns using a relatively small number of genes can be used to generate scores that are associated with probability of long-term survival in breast cancer. Oncotype-DX is one such molecular assay, which includes just 21 genes. While molecular subtypes are associated with distinctive clinical features and natural history, the molecular classification of a breast cancer does not predict survival.