**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.

***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

**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

***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

***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

**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

**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

***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

**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

***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

**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

***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

***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