The Royal College of Pathologists
Pathology: the science behind the cure

Molecular Pathology – Short Answer Question (Sample)

Testing for MGMT methylation has become standard of care in the workup of glioblastoma multiforme (GBM).

a) What is DNA methylation (a), which nucleotide base does methylation occur in humans (b), which enzyme is this process mediated by (c) and what is the effect of this process (d)? (4)

   a. *DNA methylation is a process by which methyl groups are added to DNA. (1)*
   b. *Methylation occurs on cytosine bases (C), forming 5'-methylcytosine at a CpG dinucleotide site. (1)*
   c. *DNA methyltransferases. (1)*
   d. *Methylation can modify the expression state of the DNA. When located in a gene promoter, it typically leads to gene silencing or reduced gene expression. (1)*

b) What is the HUGO-approved name for MGMT (a), what is its function (b) and which region of the MGMT gene is primarily methylated in gliomas? (c) (3)

   a. *O-6-methylguanine-DNA methyltransferase. (1)*
   b. *It encodes a DNA repair protein that specifically removes alkyl groups from DNA (the O^6 position of guanine) induced by alkylating agents. (1)*
   c. *Promoter region with CpG islands (98 CpGs in total) around exon 1. (1)*

c) MGMT methylation is commonly assessed by methylation-specific PCR (MSP). Describe main steps of the MSP and how it can distinguish methylated DNA from unmethylalted DNA (a), and name two modifications of MSP which are commonly used for quantitative analysis of DNA methylation in clinical setting (b). (3)

   a. *MSP is based on a chemical reaction of sodium bisulfite with DNA which converts unmethylated cytosines of CpG dinucleotides to uracil or UpG, followed by traditional PCR with primers specially designed to amplify bisulfite converted DNA. Methylated cytosines will not be converted in this process;(1)*

      Primers are designed to overlap the CpG site of interest, which allows one to determine methylation status as methylated or unmethylated. (1)

   b. *Pyrosequencing and real-time PCR, bisulphite sequencing. (1)*

d) Approximately what percentage of primary GBMs is MGMT methylated in (a), why is its testing recommended in GBM (b) and what is the molecular basis of the benefit of the test (c)? (5)

   a. *~ 40-50% (1)*
   b. *Prognostic – MGMT methylation is associated with prolonged PFS and OS in GBM patients on adjuvant therapy; (1)*

      Predictive – MGMT methylation predicts better response to alkylating agent Temozolomide. (1)
   c. *Active MGMT removes alkyl groups from DNA induced by alkylating agents e.g. Temozolomide, rescues the cell from the damage and leads to resistance to therapy. Methylation of MGMT promoter suppresses MGMT transcription, reduces its DNA repair function and leads to increased sensitivity to alkylating chemotherapy. (2)*

e) Testing of MGMT methylation is also recommended in high grade anaplastic gliomas. What is the clinical benefit of the test in this group of glioma (a), and which other molecular marker is always tested for the management of all high grade gliomas and why (b)? (3)

   a. *Methylated MGMT is a favourable prognostic marker for these patients on adjuvant therapies (but is not predictive for alkylating chemotherapy). (1)*
   b. *IDH1 and IDH2 mutation.(1)*

      The mutation is closely associated with MGMT methylation and is prognostic for longer survival in these patients treated with any of the current therapy regimes. (1)

f) Secondary GBMs in adults develop from astrocytomas of lower malignancy grade and retain all genetic changes including MGMT methylation found in the anaplastic astrocytomas. They have an improved overall survival compared with primary GBMs, thus accurate diagnosis is important. One molecular marker is considered to be diagnostic. What is name of the marker and how is it tested in routine clinical practice. (2)

   a. *IDH1 and IDH2 mutation(1)*
   b. *Tested by IHC for IDH1 R132H mutation, if negative, PCR based testing performed for rare IDH1 and IDH2 mutation; (1)*