MGMT Promoter Methylation Conferring Sensitivity to Alkylating Agents

The UNC Hospitals Molecular Genetics Laboratory performs DNA pyrosequencing to determine promoter methylation status of the *O^6-methylguanine methyltransferase* (*MGMT*) gene. Promoter methylation is associated with low levels of MGMT protein and accompanying increased sensitivity of brain tumors, specifically glioblastomas, to alkylating agents such as BCNU (carmustine)[1]. Low levels of MGMT also appear correlated with prolonged progression-free survival (PFS) in patients with gliomas treated with temozolamide[2].

Biology of the process: MGMT is a DNA repair enzyme that is associated with tumor resistance to alkylating agent therapy[3]. MGMT rapidly reverses alkylation, including methylation, at the O^6 position of guanine by transferring the alkyl group to the active site of the enzyme[4]. Lack of MGMT in the cell allows accumulation of O^6-alkylguanine in the DNA which, following incorrect pairing with thymidine, triggers mismatch repair, inducing DNA damage signaling and cell death[5]. Lack of MGMT expression is due to methylation of a CpG island located in the 5' region of *MGMT* (bp -552 to +289) which includes 97 CpGs[6]. Methyl-CpG-binding proteins will bind to aberrantly methylated sequences which leads to alterations in chromatin structure, thus preventing the binding of other transcription factors, effectively silencing *MGMT*[6].

Clinical indications for *MGMT* promoter methylation testing: Patients newly diagnosed with high grade gliomas (anaplastic astrocytomas and glioblastomas) or patients with gliomas who are being considered for temozolamide therapy.

Laboratory testing for *MGMT* promoter methylation: The preferred sample is a paraffin block containing at least 50% malignant cells or five unstained slides, 4- 8 uM thick on plain glass, plus an H&E stained slide and a copy of the surgical pathology report. Tumor cells are enriched by macro-dissection, if needed, and the extracted DNA is subjected to a bisulfite-treatment step followed by pyrosequencing to determine methylation status of selected sites in the CpG island of the *MGMT* promoter. Results are interpreted by a pathologist. Turn-around time for results is expected to be two weeks.

1 Belanich, M. et al., Retrospective study of the correlation between the DNA repair protein alkyltransferase and survival of brain tumor patients treated with carmustine. *Cancer Res* **56** (4), 783 (1996).

2 Hegi, M. E. et al., MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* **352** (10), 997 (2005).

3 Gerson, S. L., MGMT: its role in cancer aetiology and cancer therapeutics. *Nat Rev Cancer* **4** (4), 296 (2004).

4 Pegg, A. E., Repair of O(6)-alkylguanine by alkyltransferases. *Mutat Res* **462** (2-3), 83 (2000).

5 Ochs, K. and Kaina, B., Apoptosis induced by DNA damage O6-methylguanine is Bcl-2 and caspase-9/3 regulated and Fas/caspase-8 independent. *Cancer Res* **60** (20), 5815 (2000); Stojic, L. et al., Mismatch repair-dependent G2 checkpoint induced by low doses of SN1 type methylating agents requires the ATR kinase. *Genes Dev* **18** (11), 1331 (2004).

6 Weller, M. et al., MGMT promoter methylation in malignant gliomas: ready for personalized medicine? *Nat Rev Neurol* **6** (1), 39.

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