

LETTER to the EDITOR

Targeted Bisulfite Sequencing-Based Analysis of *MGMT* Promoter Methylation in Meningiomas*Asian Pac J Cancer Prev*, 17 (5), 2727-2728**Dear Editor**

Currently we are working on the role of aberrant DNA methylation in pathogenesis and progression of meningiomas - brain tumors frequently diagnosed in adults. For this reason we read an article by Larijani et al. published in APJCP Vol 15, 2014 with a special interest. This article concerns the role of promoter methylation of *MGMT* gene promoter in benign and malignant meningiomas. The authors reported high frequency of this epigenetic abnormality in meningeal tumors, especially in atypical and anaplastic meningiomas (Larijani et al., 2014). These two subtypes are less frequent than benign meningiomas but they represent an important clinical problem due to the high ratio of tumor recurrence and low survival rate.

The incidence of *MGMT* promoter methylation provide a potential clinical usefulness as this epigenetic abnormality is an important predictive factor for chemotherapy with temozolomide (TMZ) in other brain tumors - gliomas. *MGMT* promoter methylation is probably the best known and validated epigenetic biomarker that has been implemented in molecular diagnostics and is expected to be included in the new, oncoming WHO classification of CNS tumors (Dullea and Marignol, 2015).

The results by Larijani et al. (2014) suggest that a large proportion of meningioma patients could benefit from TMZ based treatment, similarly to glioma patients. The early trial of chemotherapy with TMZ in meningioma patients was rather disappointing, however this trial included only 16 patients with tumors refractory to standard treatment who doesn't undergo any preselection (Chamberlain et al., 2004).

By the use of Pubmed search we identified 6 additional

reports on *MGMT* promoter methylation status in meningiomas. All the studies were focused on DNA methylation of the same genomic region at 5'UTR of *MGMT* gene, that was proved to be clinically relevant in clinical trial on the usefulness of TMZ in gliomas (Hegi et al., 2005). Very similar methodology was applied for *MGMT* testing in these studies - methylation-specific PCR (MSP) and 6 out of 7 previous studies utilized the same PCR primer sequences. Despite the technical similarity the particular results are very inconsistent and the observed frequency of *MGMT* promoter methylation ranged from 0 to 34%, as shown in Table 1. It can't be excluded that some ethnical, demographical differences between the examined populations could influence the discrepancy of the reported results, but it is also highly probable that technical details in *MGMT* testing played there a major role.

MSP was developed 20 years ago. It is based on the complementarity of PCR primers to bisulfite treated DNA, that depends on the presence of methylation at CpG dinucleotides in DNA (Herman et al., 1996). The sensitivity and specificity of this method strongly depends on variable technical factors like bisulfite conversion rate and PCR conditions, including temperature profile, the use of particular DNA polymerase and PCR primers. Additionally, most of studies on *MGMT* promoter methylation were done on formalin-fixed paraffin-embedded (FFPE) tissue whereas PCR reaction may be also affected by the poor quality of DNA samples. Techniques alternative to MSP were also developed and the methodology of *MGMT* testing is matter of a debate. Currently, no consensus for the gold standard for *MGMT* promoter methylation testing has been established (Dullea and Marignol, 2015).

Recently we completed the results from DNA

Table 1. List of Reports on *MGMT* Promoter Methylation in Meningiomas by MSP (Methylation Specific PCR) and qMSP (Quantitative Methylation-Specific PCR)

| Authors | <i>MGMT</i> methylation frequency | Number of Patients | Number of tumor samples | Method | PCR amplicon position (hg19) |
|--------------------------|-----------------------------------|--------------------|-------------------------|--------|-------------------------------|
| Bello M et al. 2004 | 16% (16/98) | 95 | 98 | MSP | chr10:131,265,517-131,265,596 |
| Liu Y et al. 2005 | 6% (3/48) | 48 | 48 | MSP | chr10:131,265,517-131,265,596 |
| De Robles P et al. 2008 | 0% (0/36) | 36 | 36 | MSP | chr10:131,265,517-131,265,596 |
| Brokinkel, B et al. 2010 | 1% (1/55) | 30 | 55 | MSP | chr10:131,265,497-131,265,618 |
| Aydemir F et al. 2012 | 11% (4/36) | 36 | 36 | MSP | chr10:131,265,517-131,265,596 |
| Raheleh J et al. 2014 | 0% (0/213) | 230 | 230 | q-MSP | chr10:131,265,517-131,265,596 |
| Larijani L et al. 2014 | 34% (21/61) | 61 | 61 | MSP | chr10:131,265,517-131,265,596 |

methylation profiling of different selected genes' promoters in meningiomas with the use of targeted bisulfite sequencing (TBS). The high methylation frequency of MGMT promoter reported by Larijani L et al. as well as discrepancy of the different results in literature prompt us to include MGMT into our targeted DNA methylation profiling and to assess the methylation level of this gene with alternative, new methodological approach.

TBS utilize bisulfite conversion, multiplex PCR amplification, library construction and next-generation sequencing of the PCR amplicons. This technique is currently one of the most precise methods for DNA methylation analysis of a defined genomic regions. It allows for highly accurate quantitative determination of DNA methylation ratio at each CpG included in the PCR amplicon. It also provide a precise control for bisulfite DNA conversion (Lee et al., 2013; Masser et al., 2015). We used the Targeted Bisulfite Sequencing service from Zymo Research.

We analyzed DNA isolated from FFPE tissue with QIAmp DNA Mini Kit (Qiagen) from 42 meningioma patients including 28 benign (grade I), 9 atypical (grade II), 5 anaplastic (grade III) meningiomas as well as 5 sections of normal meninges.

The region covering MGMT 5' UTR (chr10:131,265,495-131,265,627; hg19) was bisulfite sequenced for these samples with an average coverage of 6729 reads per region (range 19 – 42377 reads). All the samples including tumor sections and normal meninges were generally unmethylated and showed average DNA methylation $\leq 1\%$. High DNA methylation (over 75%) was observed only for single CpGs within MGMT promoter region in five tumor samples. However, the average methylation level for the entire region was very low in these samples. The complete detailed results are presented in Supplementary table (available at http://nauka.coi.pl/info/article/COI352a75599eff4e5ab10a5fa5c8fff50b/A?lang=en&r=publication&affil=&cid=2172#.VyNXz_mLSCh).

Our results indicate the lack of MGMT promoter methylation in meningiomas and therefore lack of its clinical usefulness as a possible biomarker. As the results were obtained with the use of new and more reliable technique as compared to previous methodology we believe it provides an interesting commentary to results by Larijani L et al and other authors.

Acknowledgements

This work was supported by the National Science Centre grant UMO-2011/01/D/NZ5/02798.

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