Methylation of MGMT in Peruvian cases of Glioblastoma: Validation of epigenetic technique

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O6-methylguanine-DNA methyltransferase (MGMT) is a key enzyme in the base-excision pathway of DNA repair (BER). MGMT remove mutagenic and citotoxic adducts from O6-guanine in DNA, the preferred point of attack for alkylating drugs in the treatment of glioblastoma. Epigenetic silencing of the MGMT gene has been associated with improved survival in several studies of gliomablastoma patients treated with alkylating agent therapy. However, procedures in brain tissues has different features regarding other body organs and gene evaluation in it requires to develop experience the field.
We implemented an approach for performing this evaluation with best acquracy. First, we perform the DNA extraction from seven frozen and formaldehyde fixed-paraffin embedded (FFPE) tumor samples (ID samples: 43, 45, 46, 48, 49, 66 and 80) and 18S and GADPH housekeeping genes evaluation by PCR. 18S and GADPH detection was positive for all frozen and FFPE samples with a concentration of 100ng. However, we found DNA degradation and loss of intensity bands in the FFPE samples. MGMT promotor methylation status was tested in 50 FFPE samples. Unmethylated (UM), methylated (M) and partial methylated (PM) with both unmethylated and methylated alleles was found in 29 (55.77%), 15.38% (n=8) and 15 (28.85%), respectivelly. We send two of our FFPE samples to Unidad de Investigación de Patología Molecular, Hospital Virgen de la Salud, Toledo, Spain, in order to evaluate the reproducibility of our methodolgy. The results were similarly, found one U sample (ID: 3) and one PM sample (ID: 42). We evaluated expression levels of MGMT gene through RT-PCR technique in 17 of the previouslly evaluated FFPE samples and we found high MGMT expression in the 7 U samples and loss of MGMT expression in the 3 M samples. The 7 PM samples showed different level of MGMT expression. Finally, we performed survival analysis and found statistically significant association between MGMT promotor methylation status and the variables of progression-free survival (PFS) and overall survival (OS). Using cox model, analysis found recurrence risk associated with PFS and death risk associated with OS in unmethylated tumor samples. Although we didn´t find statistically significant association with radiotherapy and chemotherapy at studied tumor samples, possibly to sample size (n=50). Conclusion: Evaluation of gene expression in brain samples has special features but evaluation of MGMT methylation in Latina population is posible and could predict outcome.