# STUDY OF BIOLOGICAL EPIGENETIC PATTERNS IN RESPONSE TO CHEMOTHERAPY TREATMENT OF MULTIFORM GLIOBLASTOMA

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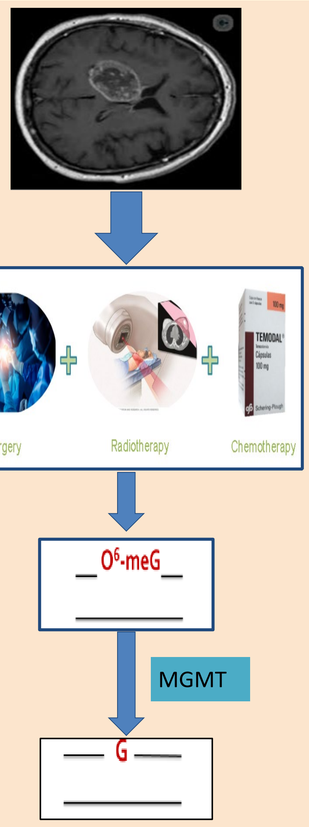
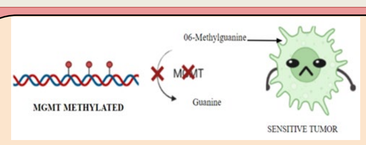
## INTRODUCTION

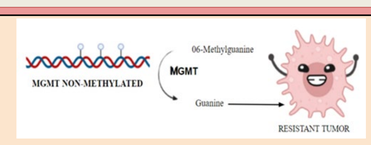
Glioblastoma multiforme (GBM) is an aggressive form of brain cancer with a low survival rate, because of the chemotherapy´s resistance which is used in combination with radiotherapy as first-line treatment after surgery. Tumour appearance and development is associated to dysfunction of the DNA repair mechanisms, changes of epigenetic patterns and deregulation of genetic expression. It has been described that DNA repair mechanisms contribute to chemotherapy resistance in GBM, so genes which are implicated in DNA repair could be used such as predictive biomarkers of the treatment´s response in GBM.

The main drug used as GBM treatment is Temozolomide (TMZ) which is an alkylating agent. TMZ induces DNA lesions such as O6-methylguanine which could be repair by O6-methylguanine methyltransferase (MGMT). The aim of this study is to analyse MGMT methylation pattern on GBM cell lines and the relationship between methylation status and response to the TMZ treatment.




## HYPOTHESIS




Being this project closely focused on the field of the epigenetic study forward the response of the tumor to chemotherapy, there will be used as a main variable the gene MGMT, capable of reversing the damages caused within DNA by TMZ.

The main hypothesis believed previously to experimentation is the fact that MGB cancer is sensitive to TMZ as long as the gene MGMT is NOT expressed within the patient's genome. In turn, the expression of MGMT is thought to be the reversive effect of those damages which would induce the cancer cell's death, and by the same token, being expressed the cancer will be resistant to treatment.

The following **objectives** suitable to this project are derivate, which are consistent to the previous hypothesis:

- To study biomarkers which may predict the responses to chemotherapy in MGB
- To analyze the epigenetic patterns of specific genes of reparation of DNA
- To observe the methylation status of specific epigenetic marks so as to know the expression of those proteins which reverse the tumoral treatment.

## MATERIALS AND METHODS

### A) SAMPLES

**A. LN229** **B. LN18**

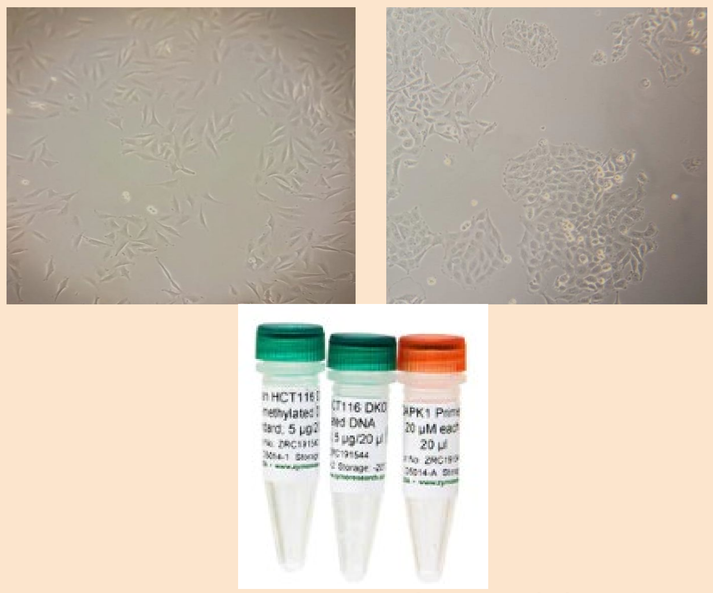


**FIGURE 1:**Genomic DNA of LN229 (A) and LN18 (B) cell lines; human DNA not methylated and human DNA methylated.

### B) STUDY OF DNA METHYLATION THROUGHOUT SODIUM BISULFITE MSP

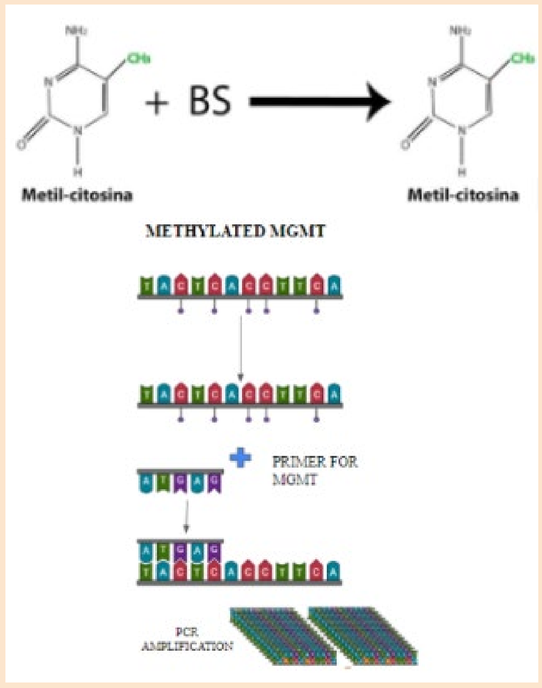



**FIGURE 2**: Non-methylated cytosines remains equal. Hence, DNA will be amplified by PCR due the bases' **chemical differences.**

Methylation of Cytosine is an epigenetic mark which reveals the state of methylation of DNA

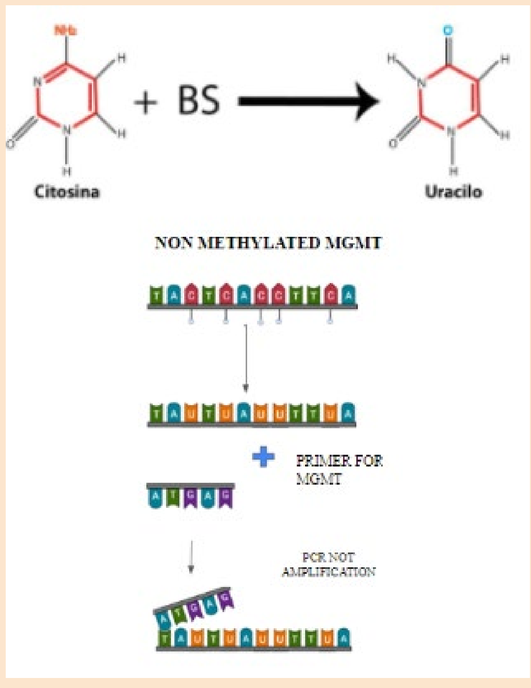



**FIGURE 3:** Sodium Bisulfite transforms methylated cytosine into Uracil owing to their **chemical similarity**.

## RESULTS

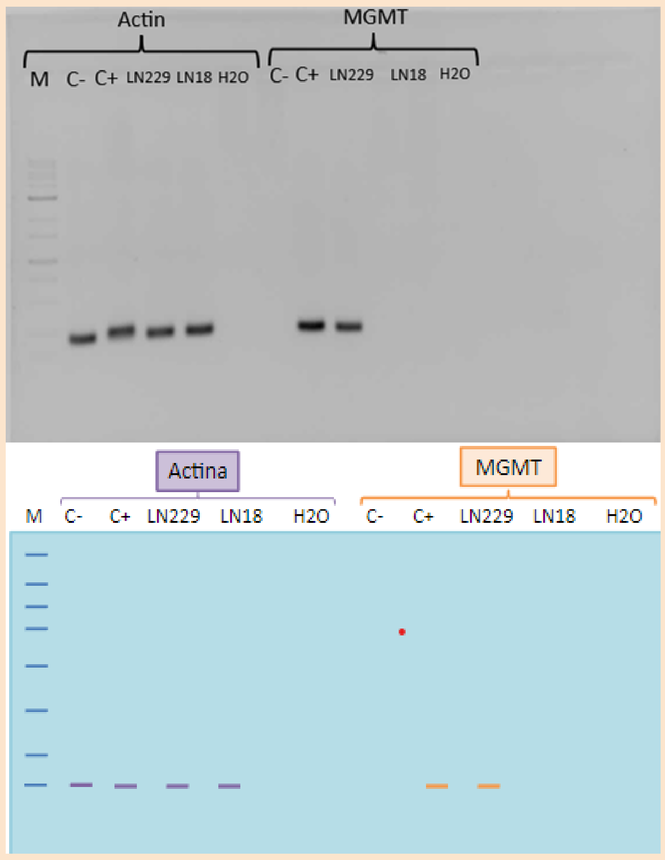



**Figure 4.** Electrophoresis in 0,7% agarose gel.Study of the obtained samples by MSP using genetic primers specific for genes: Actin and for gene MGMT.

Practical results subsequent to experimentation.

**Figura 5.** Electrophoresis in 0,7% agarose gel. Study of the obtained samples by MSP using genetic primers specific for genes: Actin and MGMT.

Theoretical results previous to experimentation.

## CONCLUSIONS

- The result of PCR is reliable: there is not amplification in neither of the samples which do not contain DNA (referred as "$H_2O$" in figure X).
- DNA modified with sodium bisulfite is in optime conditions. All DNA samples show amplification in MSP when Actin primers were used.
- In MGMT case, primers allow to distinguish DNA methylated (C+) and DNA not mutilated (C-). Therefore, primers are suitable to the aim of this study.
- LN229 DNA show amplification for MGMT (primers for methylated DNA fit to it). Consequently, presence of protein MGMT is not expected. Hence, that cellular line is sensitive to temozolomide.
- LN18 DNA does not show amplification for MGMT gen. LN18 does not have MGMT DNA methylated and MGMT protein are not expected. Tumor cells will present resistance to temozolomide as a result.