STUDY OF EPIGENETIC BIOMARKERS IN RESPONSE TO GLIOBLASTOMA TREATMENT

AUTORES

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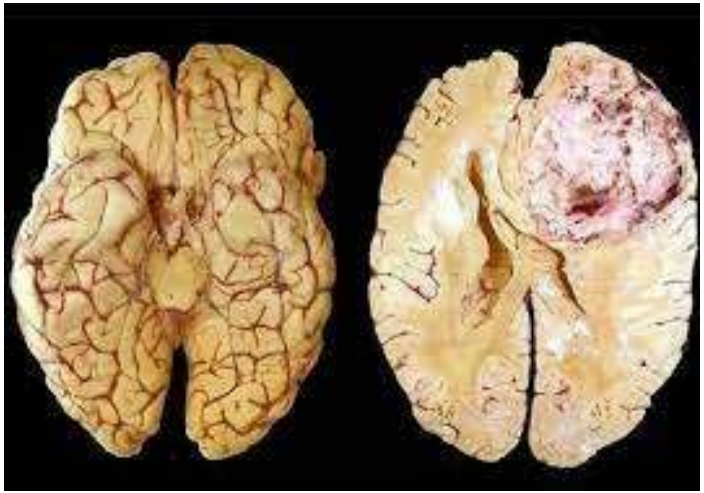
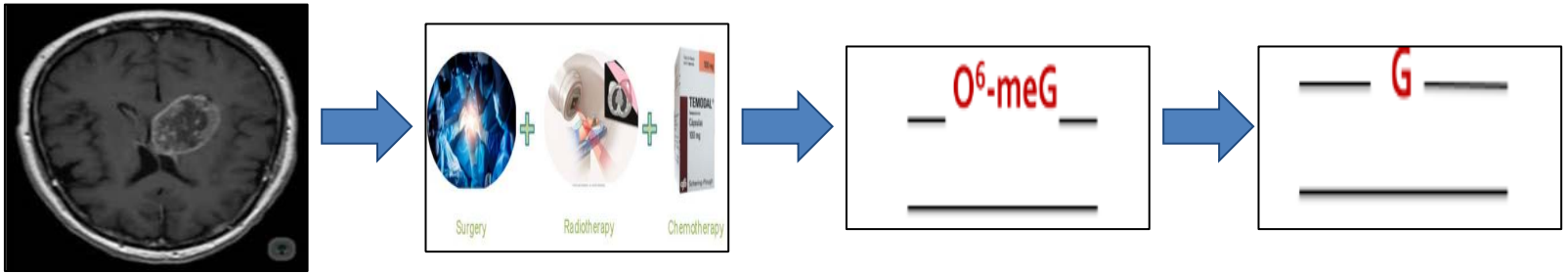
1. INTRODUCTION

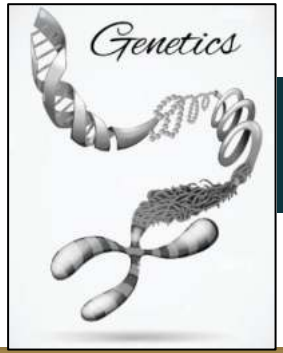


- Aggressive brain tumor
- Surgery + radiotherapy + chemotherapy
- DNA damages owing to chemotherapy —either repared or not

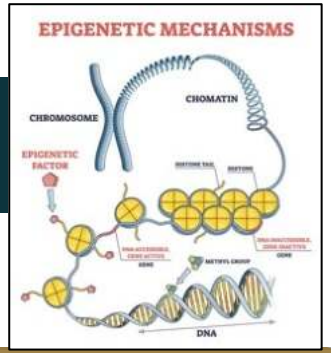
INTRODUCTION



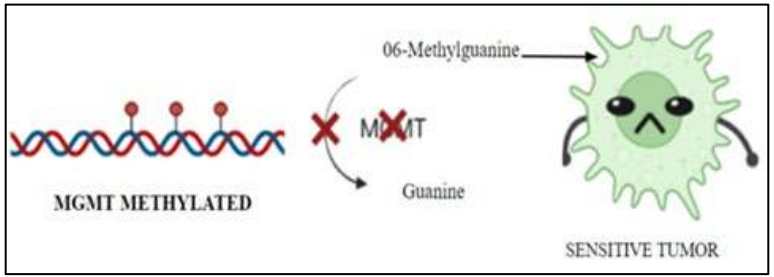
- Tumour appearance and development ⟶ dysfunction of the DNA repair mechanisms
- DNA repair mechanisms contribute to chemotherapy resistance in GBM
- Chemotherapy ⟶ TEMOZOLOMIDE (TMZ)
- MGMT ⟶ METHYL GUANINE METHYL TRANSFERASE

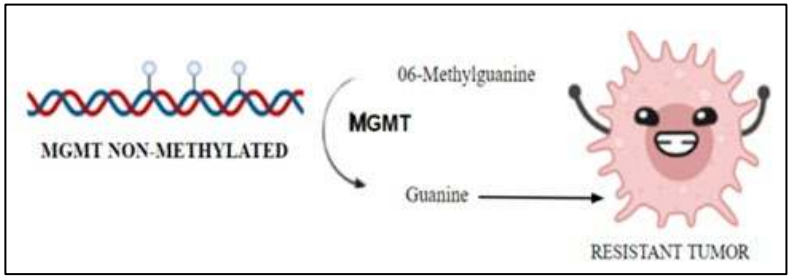


COMBINATION OF GENETICS & EPIGENETICS



2. HYPOTHESIS





- Focus: epigenetics field ; study of tumoral responses to chemotherapy
- Main variable: gene MGMT, capable of reversing the damages caused within DNA by TMZ.

- HYPOTHESIS 1: MGB sensitive to treatment (TMZ) → MGMT NOT EXPRESSED
- HYPOTHESIS 2: MGB resistant to treatment (TMZ) → MGMT EXPRESSED

2. OBJECTIVES

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.

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MATERIALS AND METHODS

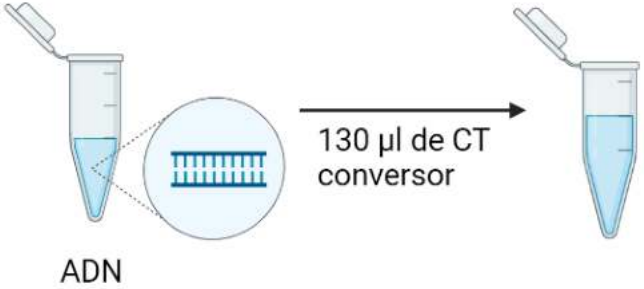
CELLULAR LINES:

POSITIVE CONTROL OF METHYLATION:
DNA completely methylated

LN229:
Cellular line of Glioblastoma

NEGATIVE CONTROL OF METHYLATION:
DNA completely non methylated

LN18:
Cellular line of Glioblastoma



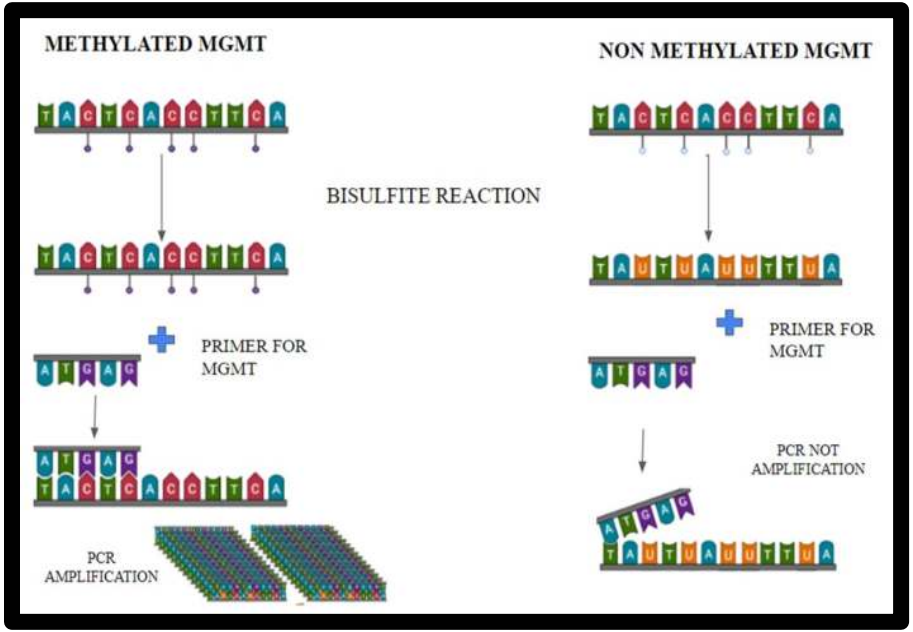
Termociclador:
- 98° 10 min
- 64° 2'30 h
- 4° ∞

Muestra 1: Control negativo
Muestra 2: Control positivo
Muestra 3: LN229
Muestra 4: LN18

SODIUM BISULFITE TREATMENT

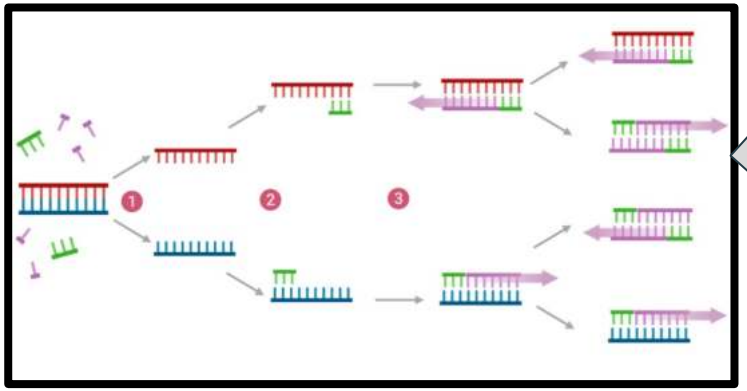
Sodium bisulfite DNA treatment allows for discrimination between methylated and unmethylated cytosines .

1. The MGMT genes non-methylated, in presence of bisulfite, contain uracil in their sequence of nucleotides instead of cytosine.
2. The sequences of methylated MGMT conserve the chemical structure of cytosine.
3. MGMT genes methylated will not concord with the PRIMER input for MGMT established for the PCR amplification, and DNA will not replicate.



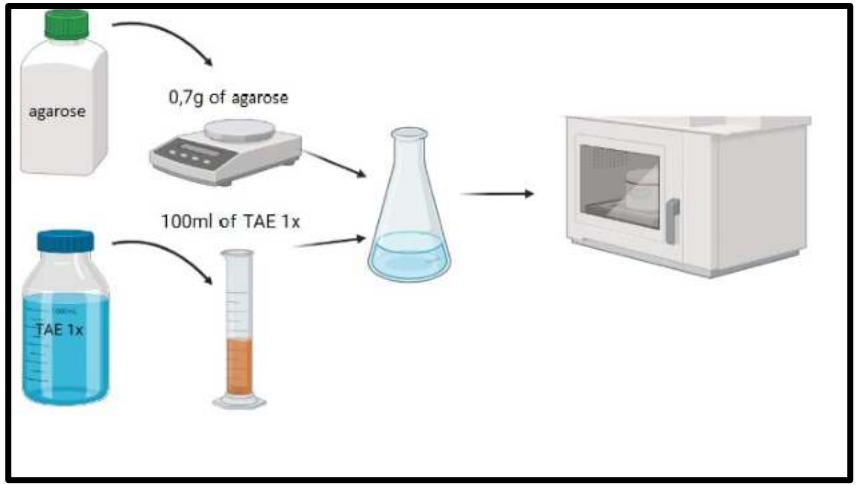
PCR

5 PCR reactions were carried out, 4 of them according to the cellular lines, and another one with water for making sure DNA has not been contaminated during the process.



Electrophoresis

An agarose gel has been prepared with the aim of analyzing the products from the PCR, to determine the MGMT methylation state.



Informatic programmes

In order to visualize the gel and obtain the results from the electrophoresis, we used the program associated to transilluminator device, which shows us if the DNA is methylated or not.

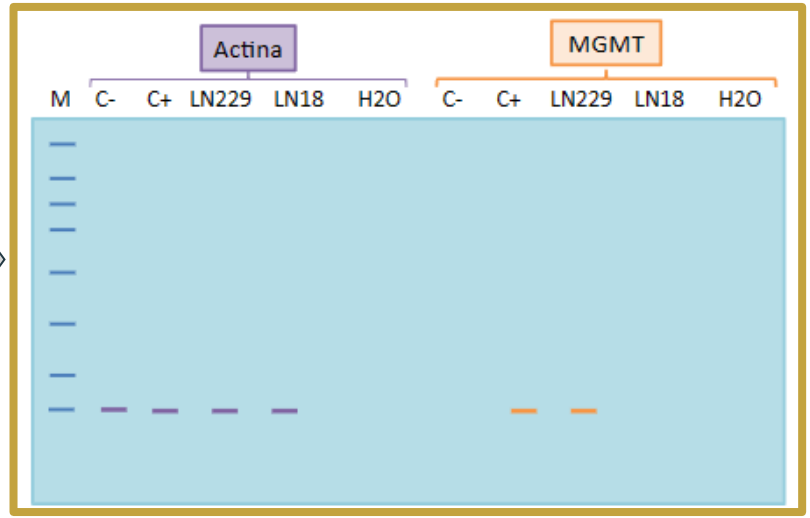
Results obtained through transilluminator:

Experimental design:

From Genomic DNA, which had before been extracted from specific cellular line of MGB, it was performed a treatment with bisulphite as an epigenetic mark so as to know the state of methylation of DNA.

Afterwards, it will be performed by an amplification by PCR of this genetic material.

Those MGMT genes non methylated will not be replicated whilst methylated ones, will.



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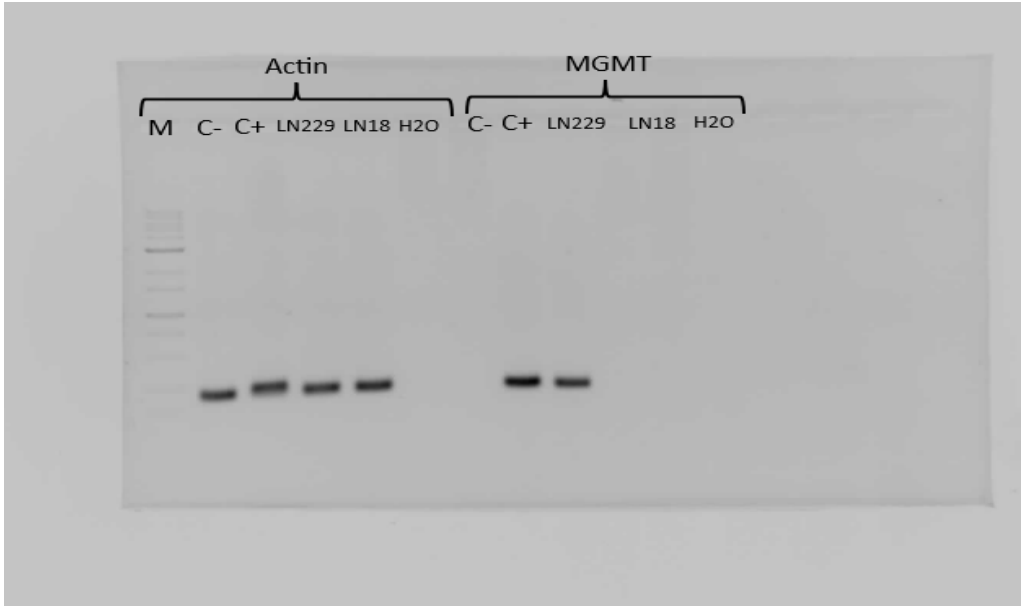
RESULTS

RESULTS



Agarose gel electrophoresis using actin protein as concurrent control for knowing which cell lines be resistant or sensitive to chemotherapy:

- **LN229**
  - it shows amplification
  - not presence of MGMT
  - sensitive to chemotherapy
- **LN18**
  - does not show amplification
  - presence of MGMT
  - resistant to chemotherapy

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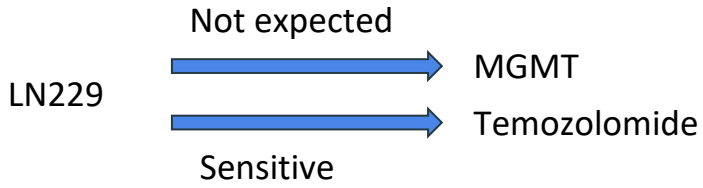
CONCLUSIONS

Conclusions

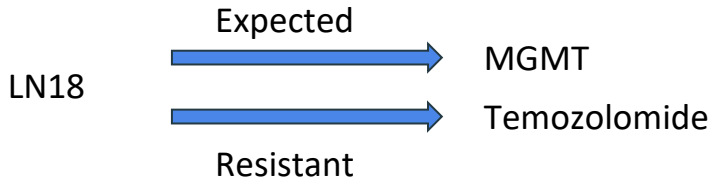
1. There is no amplification in either of the samples which do not contain DNA.
2. DNA modified with sodium bisulfite is in optime conditions. All DNA samples show amplification in MSP when Actin primers were used.
3. In MGMT case, primers allow to distinguish DNA methylation and DNA non methylated.

Conclusions

5. LN229 DNA shows amplification for MGMT.



6. LN18 DNA doesn't show amplification for MGMT gen.



ACKNOWLEDGEMENTS

Thanks for your attention.

CES LOPE DE VEGA

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Epigenética y
Reparación del ADN