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INSTITUTO MAIMÓNIDES DE INVESTIGACIÓN BIOMÉDICA DE CÓRDOBA

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ROLE OF DNA REPAIR PROCESSES IN RESPONSE TO CHEMOTHERAPY IN GLIOBLASTOMA

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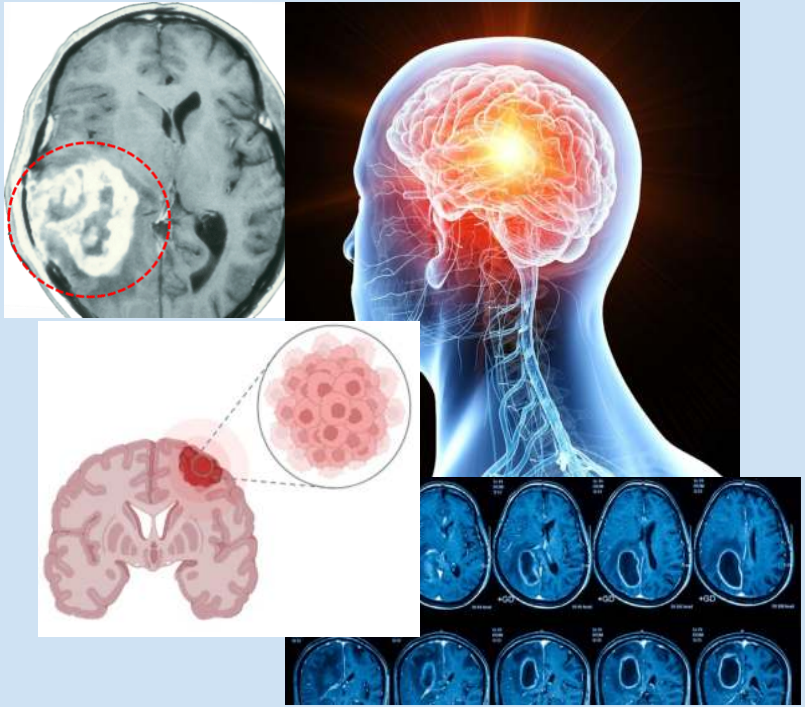


1.INTRODUCTION

GLIOBLASTOMA

- ➔ Central Nervous System tumor → glial tissue
- ➔ Higher incidence between 45 → 65 years

TEMOZOLOMIDE

Alkylating agent + methyl group
Damaging tumor cells

TREATMENT

- ➔ Surgical resection
- ➔ Radiotherapy
- ➔ Chemotherapy

} <u>Temozolomide</u>

RESISTANCE TO TREATMENT

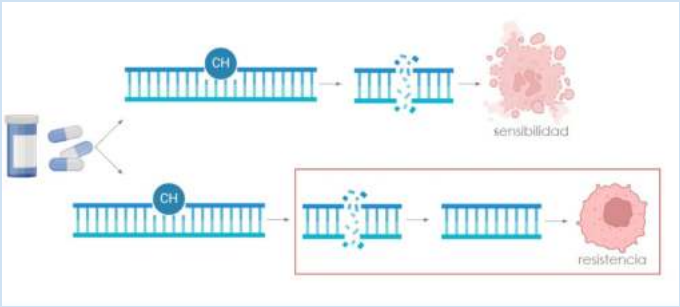
- ➔ Low survival rate
  Resistance of the tumor cells
- ➔ It repairs the damage caused by **temozolomide**
  O6 methylguanine.

HYPOTHESIS

We started from two cell lines: **A172** and **T98G**

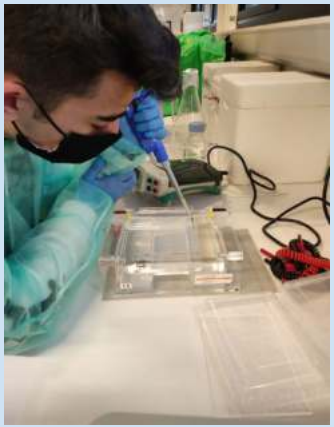
➔ We expect that the A172 line will not express MGMT since its component cells are sensitive to temozolomide.

➔ On the other hand, for the T98G line, the opposite is expected, since it is made up of cells resistant to treatment, therefore they would not kill the cancer



2.OBJECTIVES

- ➔ Purify mRNA from two glioblastoma cell lines.
- ➔ Obtain cDNA by reverse transcription.
- ➔ PCR amplify the MGMT gene.
- ➔ Analyze the expression of the MGMT gene.



MATERIALS AND METHODS

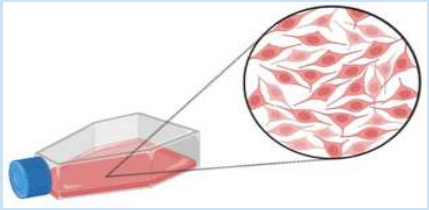
CELL CULTURE

RNAm PURIFICATION AND MEASURE IN NANODROP

We start from two celular lines of glioblastoma:

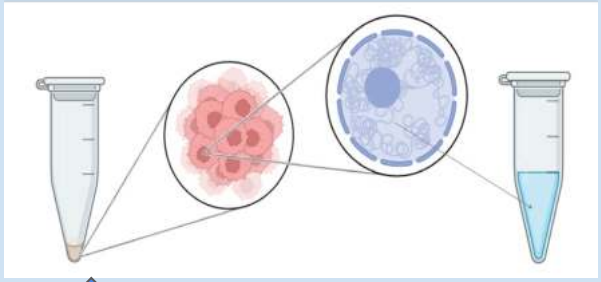
- A172
- T98G

- These cellular lines are adherent, so they grow in a monolayer inside culture jars.



- Lifting up and centrifuging these cells, cellular pellets are obtained.

From these cellular pellets, the purification of the RNAm from the tumoral cells is done.



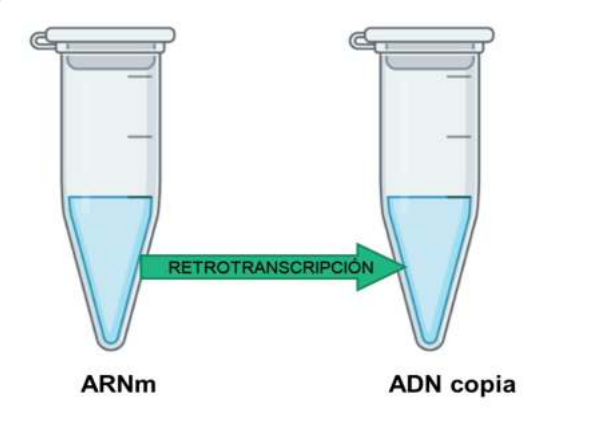
To know the concentration of RNAm in each sample, and the quality of the results, we will use

NanoDrop

MATERIALS AND METHODS

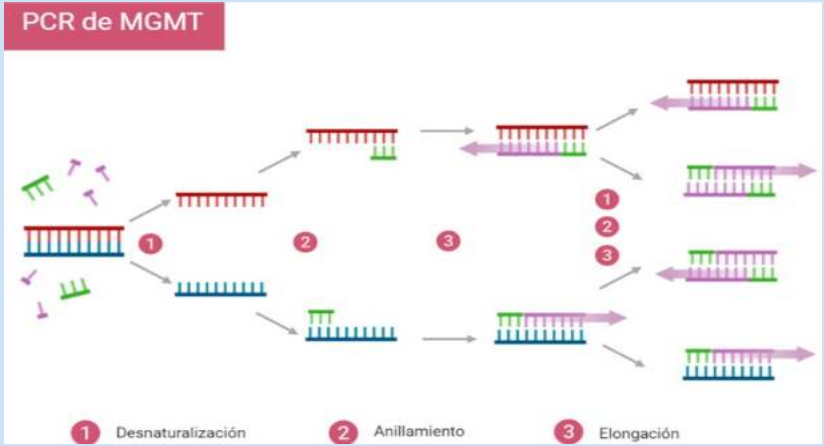
RETROTRANSCRIPTION TO DNAc

The RNAm is subject to a retrotranscription process, to obtain the DNAc of both cellular lines.



PCR

To analyse the presence or absence of the gen MGMT, that codifies the enzyme MGMT inside the glioblastoma cells, a PCR is used to amplify a fragment of DNA.

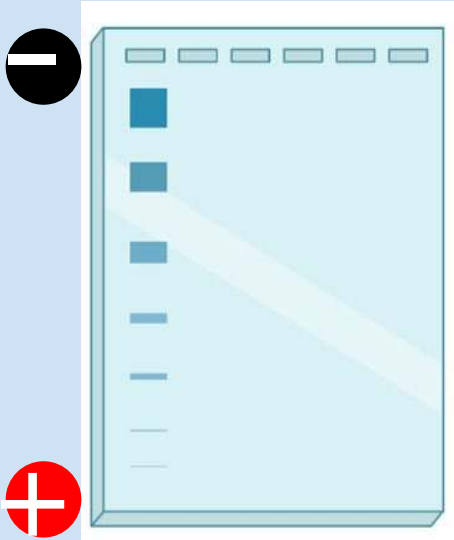


MATERIALS AND METHODS

ELECTROPHORESIS

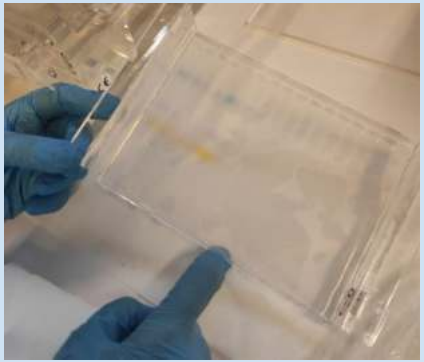
- Electrophoresis is a technique used to separate DNA, RNA, or protein molecules based on their size and electrical charge.

- An electrical current is used to move molecules through a gel. The pores inside the gel allows smaller molecules to travel faster than larger ones.



That process was used to separate the PCR product from the DNAc.

After, that results are visualize in the transilluminator, comparing the expression of the MGMT gene in sensitive (A172) and resistant (T98G) glioblastoma cells.



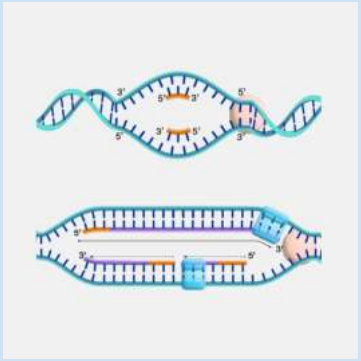
EXPERIMENTAL DESIGN

From the **A172** and **T98G** cell lines isolated from patients with glioblastoma multiforme:

- ➔ A cell pellet was obtained by centrifugation.
- ➔ For its purification, different reagents are added to a column with a filter.

NanoDrop is then used to → determine the concentration of each mRNA sample.
→ and its quality.

At the end of this test, the mRNA must be subjected to reverse transcription to obtain the cDNA of both cell lines.



A PCR is carried out for the amplification of the MGMT gene using *specific primers.*

Finally, electrophoresis in an agarose gel, was performed to:

- ➔ separate the PCR product from the DNA copy.

This test is performed to compare the expression of the MGMT gene in sensitive (A172) and resistant (T98G) glioblastoma cells.

SOFTWARE (NANODROP)

The nanodrop program, called “SynGene” has been used to know the mRNA concentration of each sample, as well as its quality.

- ➔ The mRNA has been taken, the program has been opened and ‘’Nucleic Acid’’ has been selected at the start of the program.
- ➔ Then, water was pipetted into the nanodrop.
- ➔ Later on, the RNA measurement option was chosen and the sample to be measured was homogenized with vortex ⟶ taking 1µl of the sample was pipetted into the Nanodrop and pressed.
- ➔ Finally, each sample was measured twice and the values of the different measurements were averaged.

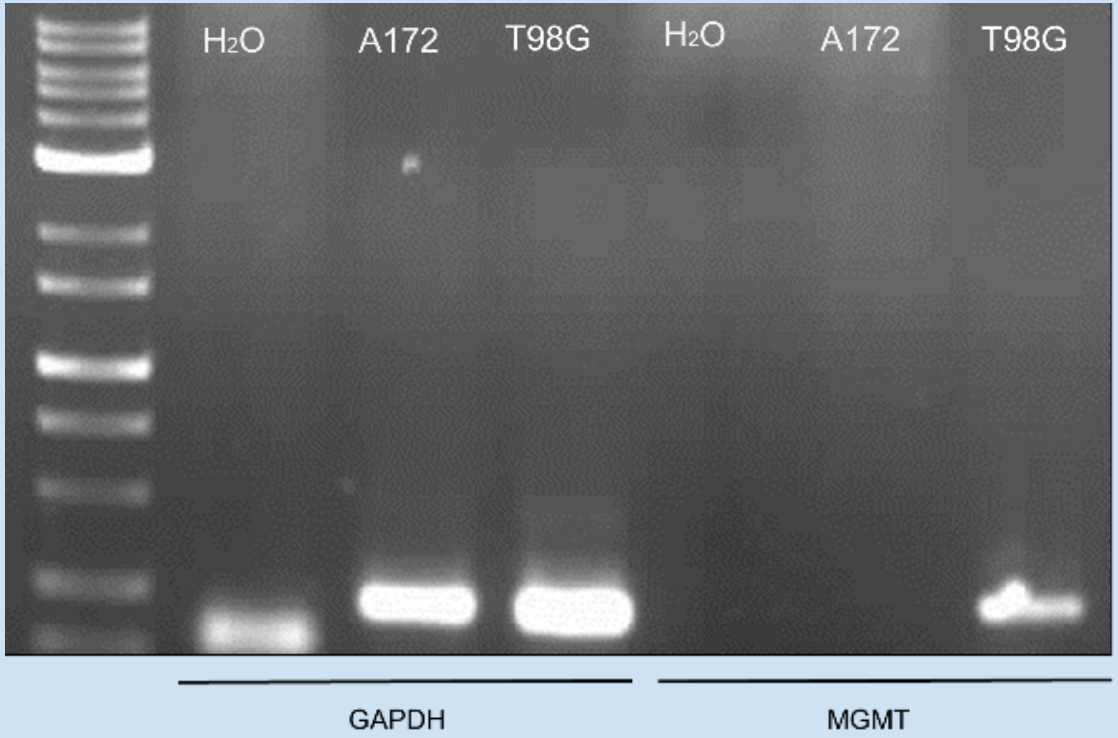
RESULTS

	260/280	260/230	CONCENTRATION
A172	1,99	1,63	185,8 ng/µl
T98G	2,04	1,61	470,85 ng/µl

RESULTS

Agarose gel electrophoresis result

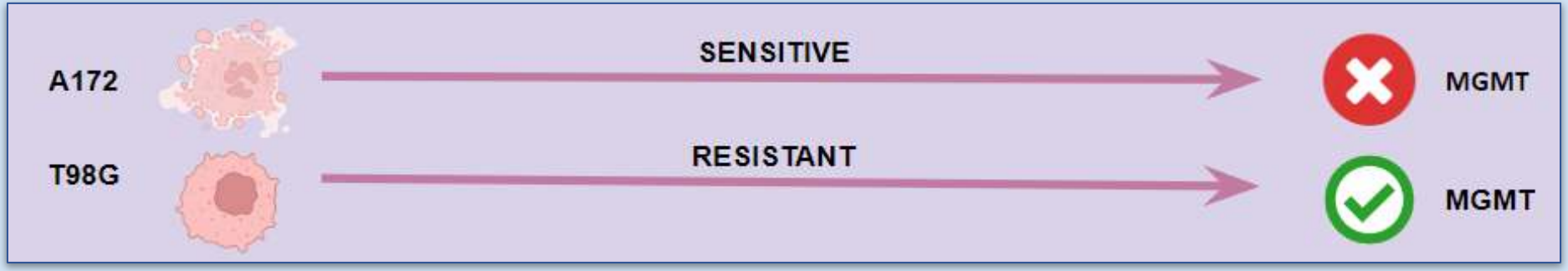
Expression of the MGMT gene is only present in the resistant line (T98G).



CONCLUSIONS

The **A172 cell line**, *sensitive* to therapy, **does not express the MGMT gene**.

The **T98G cell line**, *resistant* to therapy, **expresses the MGMT gene.**



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