ROLE OF DNA REPAIR PROCESSES IN RESPONSE TO CHEMOTHERAPY IN GLIOBLASTOMA

STUDENTS

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INTRODUCTION

GLIOBLASTOMA

It is a type of CNS tumor, formed from glial tissue. Its incidence is higher between 45-65 years; the survival rate is very low.

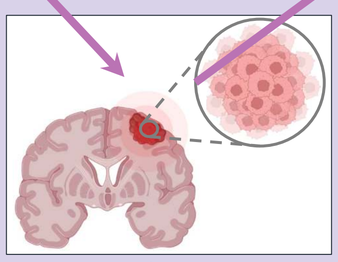


Figure 1. Glioblastoma

TREATMENT

It consists of surgical resection, radiotherapy and chemotherapy (temozolomide).

TEMOZOLOMIDE

Alkylating agent that adds a methyl group to DNA, damaging tumor cells.

RESISTENCE TO THE TREATMENT

The low survival rate is due to the resistance of the tumor cells to the treatment.

Since the resistance their offer, it repairs the damage caused by temozolomide, one of which would be O6 methylguanine.

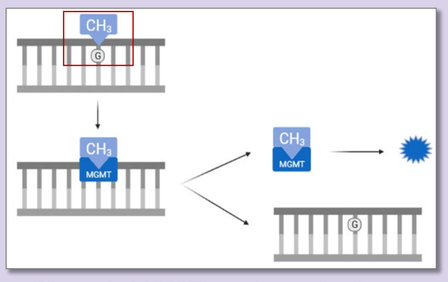


Figure 2. MGMT repair mechanisms

OBJECTIVES

1. To Purify mRNA from two glioblastoma cell lines.
2. To Obtain cDNA by a retrotranscription process.
3. To amplify MGMT gene by PCR.
4. To Analyze the expression of the MGMT gene.

MATERIALS AND METHODS

1. RNA PURIFICATION

Starting from the pellets, the RNA of both cellular lines of the glioblastoma are purified through a commercial kit.

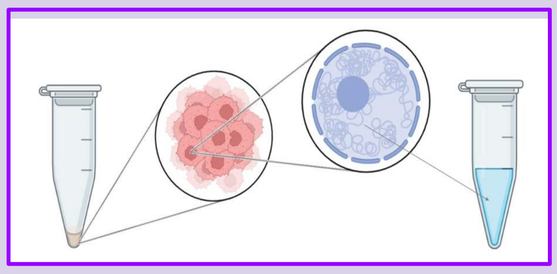


Figure 3. Diagram of the RNA purification

2. RETROTRANSCRIPTION

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

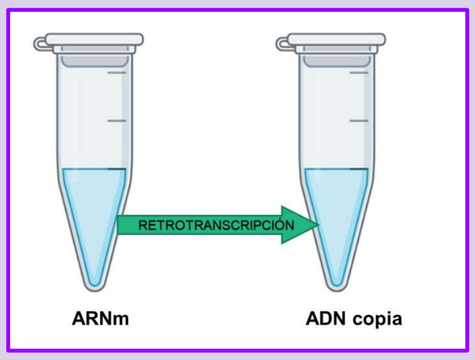


Figure 4. Retrotranscription diagram

3. PCR

The cDNA of both cell lines is amplified by PCR. The constitutive gene is GAPDH, and the problem gene is MGMT.

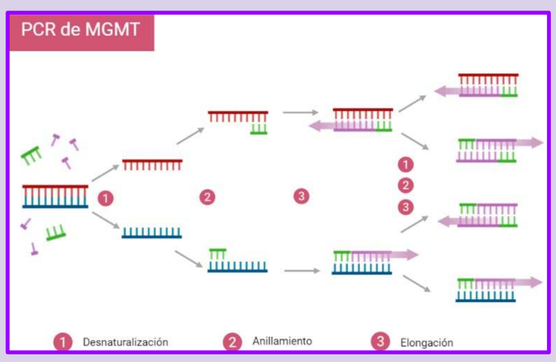


Figure 5. Polymerase chain reaction

4. AGAROSE GEL ELECTROPHORESIS

An agarose gel was performed to separate the PCR product and visualize the results on the transilluminator, comparing MGMT gene expression in sensitive (A172) and resistant (T98G) glioblastoma cellular lines..

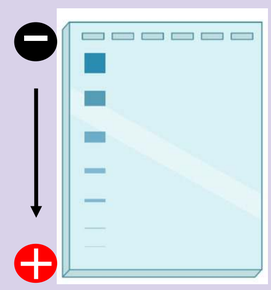


Figure 6. Agarose Gel Electrophoresis

RESULTS

RNA´s quality is valid

	260/280	260/230	CONCENTRATION
A172	1,985	1,63	185,8 ng/µl
T98G	2,04	1,61	470,85 ng/µl

Table 1. Results of Nanodrop of the RNA samples.

Data obteined from the RNA measure in Nanodrop derived from the cellular pellets of the glioblastoma cellular lines A172 and T98G.

MGMT expression is only present in the resistant line

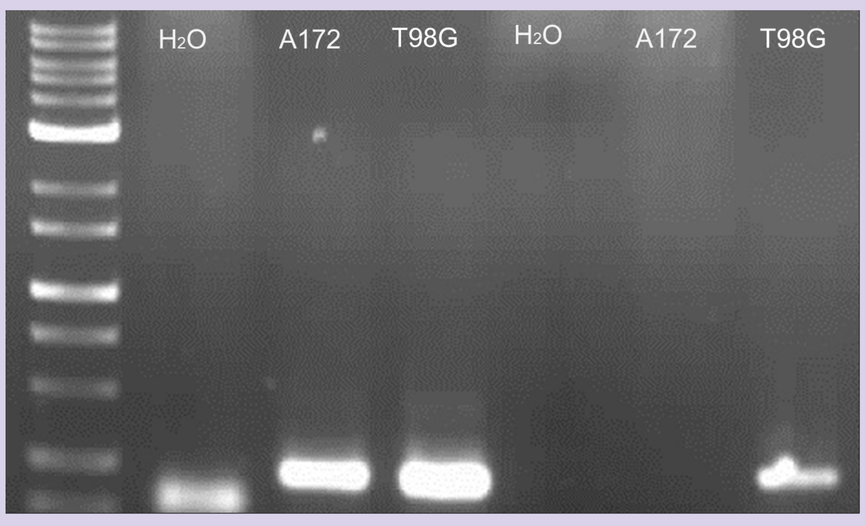
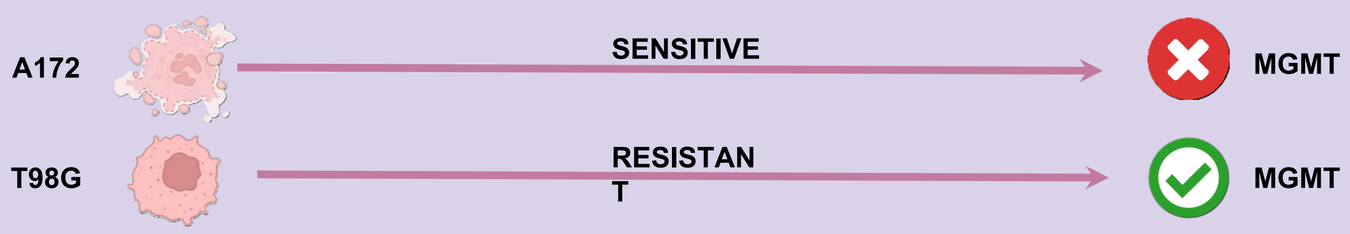


Figure 7. Agarose gel electrophoresis result

Results obtained from electrophoresis of PCR products from glioblastoma cellular lines A172 and T98G, with GAPDH and MGMT primers.

CONCLUSIONS

1. The A172 cell line, sensitive to the therapy, does not express the MGMT gene.
2. The T98G cell line, resistant to therapy, expresses the MGMT gene.



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