MGMT Status of a Rare Glioblastoma Stem Cell Line

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Abstract
Glioblastoma (GBM) is one of the most common, lethal, and aggressive type of brain tumor in adults. The standard chemotherapy for this type of tumor is temozolomide (TMZ). TMZ is an alkylating agent which is a drug that damages DNA. MGMT is a protein found in many tumors, and when the MGMT level is low in GBM, the patient is more likely to live longer. It has been proven that MGMT, an anti-alkylating agent, undoes the work of TMZ, making it harder for the alkylating agent to work. The MGMT level allows us to know which cell lines to compare in future experiments because we want to compare MGMT-high to MGMT-low cell lines in order to develop a treatment for MGMT-high tumors. We used a western blot to detect MGMT in a GBM cell line known as GSC20 in order to determine if the MGMT level is high or low. Our western blot showed no MGMT, even in positive controls. It also showed that tubulin level varies between GSC20 and T98 GBM cell lines. Therefore, we concluded that, in order to better identify MGMT-low and MGMT-high glioblastoma cell lines, we need to not only explore different anti-MGMT antibodies from different manufacturers but also use a different loading control. These two changes are essential to improve our MGMT assessment process and results.

Methods

1. T98 Lysates
2. GSC20 Lysates

Make lysates from T98 and GSC20 cells

Pipette prepared lysates into gel wells

Wash with TBST

Transfer protein to membrane

Run gel for 30 min. at 200V to separate different proteins

Soak cushions and place membrane on one side

Primary antibodies bind to MGMT and loading control protein

Cover completely due to light sensitivity and rock for 1 hour

Each secondary antibody binds to a primary antibody bound to a protein.

Wash with TBST 5x for 5 minutes

Put membrane into clear well, pour in primary antibody solution, seal, and rock for 1 hour.

Wash with TBS 2x for 3 minutes

Primary antibodies bind to proteins and help make antibody binding specific then rock for 30 minutes

Image of glioblastoma inside brain

Image from: science.org

Image from: accordhealthcare.us

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References