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Effect of tumor compaction on immune cell penetration

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ABSTRACT

The main objective of this project is to gain a better understanding of the obstacles encountered by immune cells when fighting glioblastoma tumors. An example of this is the high degree of compaction of these tumors, which hinders the penetration of cells and drugs. This phenomenon can be reversed with certain compounds, such as blebbistatin.

INTRODUCTION

Glioblastoma: Importance and Challenges

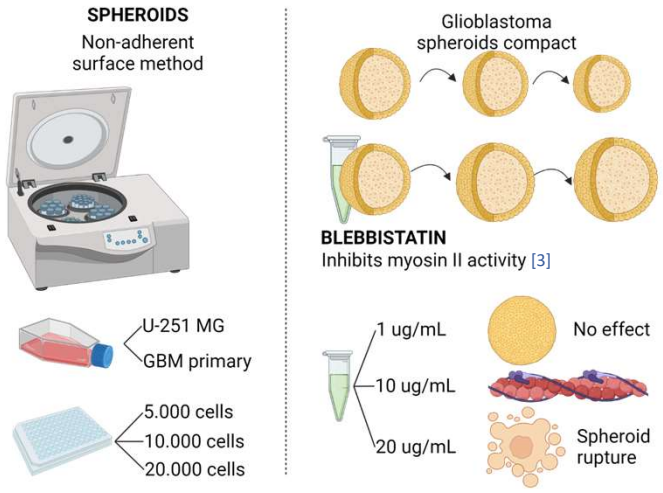
Glioblastoma is the most common type of brain cancer and one of the cancers with the highest mortality rate, primarily due to treatment resistance and high recurrence rates after surgery [1]. Furthermore, glioblastoma tumors create an unfavorable environment for immune cells, limiting their action, rendering traditional immunotherapy ineffective against this type of cancer [2]. The main objective of this project is to gain a better understanding of the obstacles encountered by immune cells in combating glioblastoma, in order to eliminate these barriers and enhance the patient's own defenses against the tumor.

Advanced Techniques

By utilizing three-dimensional cell cultures (spheroids), laboratory conditions can replicate the tumor and its environment, allowing for a better understanding of immune system inhibition mechanisms and the search for new strategies that enhance the effect of cells that defend our bodies.

The co-cultivation of these spheroids with immune cells extracted from peripheral blood enables the observation and study of the actions of these cells when in the presence of three-dimensional tumor structures, with the potential to modulate various parameters of both the spheroid itself and its surrounding environment.

OUR APPROACH



Spheroids evolution (Figure 1)

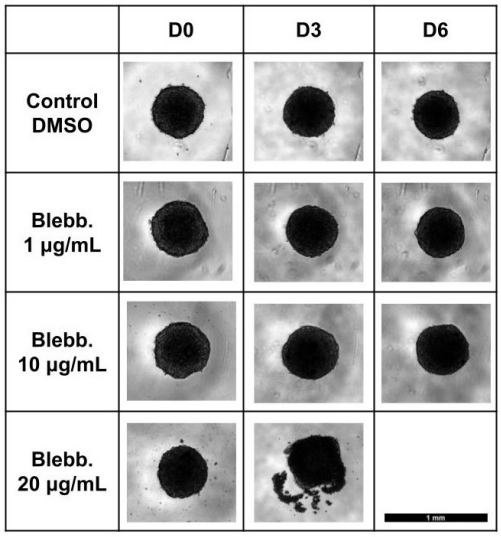


Figure 1. Spheroids evolution
Spheroids generated with 10,000 U-251 MG cells.
The experiment with the highest concentration of blebbisatin was stopped on day 3 because the spheroids were decomposing.

Effect of blebbistatin (Figure 2)

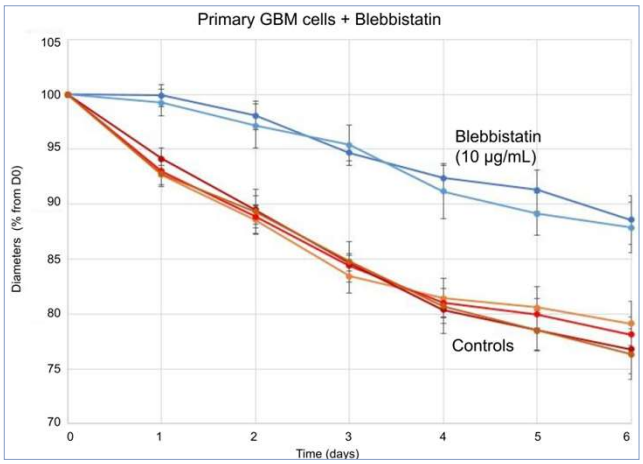


Figure 2. Effect of blebbistatin (on primary GBM cells)
Graph showing the evolution in the diameters of the spheroids

Next experiments (Figure 3)

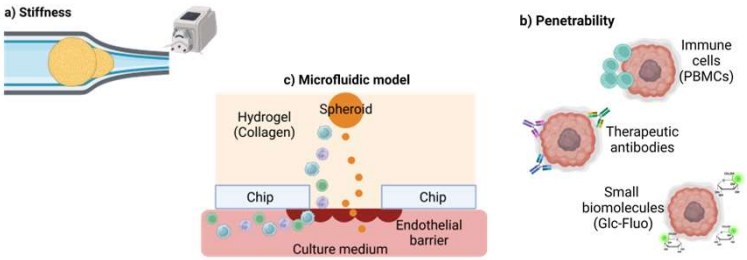


Figure 3. Next experiments
a) Stiffness assay to ensure that this is the reason of the change in sizes. **b**) Penetrability experiments to evaluate if (and how much) this parameter changes. **c**) Scheme of the future microfluidic model

CONCLUSIONS AND FUTURE WORK

Blebbistatin has been shown to be a compound capable of relaxing the intercellular forces present in glioblastoma spheroids, thereby reducing the degree of spheroid compaction.

To quantify this compaction, stiffness assays (Fig. 3a) will be conducted before and after the addition of blebbistatin.

Furthermore, by obtaining more relaxed spheroids, it may be easier for immune cells and drugs to access their interior. For this reason, the next steps following these experiments involve the addition of immune cells, antibodies, and trackable small molecules, such as fluorescent glucose, in order to compare their penetration into the spheroid's interior with and without the effect of blebbistatin (Fig. 3b).

Finally, a microfluidic model will be crafted combining the glioblastoma spheroids along with immune and endothelial cells (Fig. 3c).

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