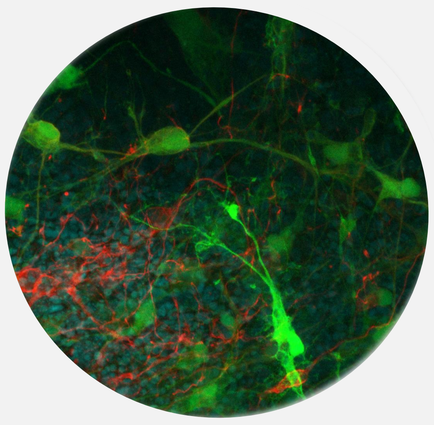

3D IN VITRO MODEL FOR THE STUDY OF THE RECIPROCAL INTERACTION BETWEEN DIFFUSE LOW-GRADE GLIOMA AND ITS MICROENVIRONMENT

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INTRODUCTION

Malignant gliomas include a diverse group of intrinsic central nervous system tumors that remain incurable.[1] Diffuse Low-Grade gliomas (DLGG) IDH1 mutate constitute approximately 15% of all gliomas,[2] and have a mortality rate of 75%, addressing the importance of understanding their molecular and cellular complexity.[3]

Currently, it is well-established that studying gliomas necessitates consideration of the tumor microenvironment (TME).

Recent research has described the critical role of direct interactions between cancer cells and the TME in tumor growth regulation.[4] For instance, it has been documented that neurons in the environment associate with glioma cells (Figure 1)[5,6] highlighting the complexity of cell-cell interactions within the glioma TME. However, these interactions, particularly for DLGG IDH1 mutated, remain unexplored. Therefore, it is important to replicate the tumor environment in vitro to gain a deeper understanding of cellular and molecular dynamics within the TME, with the aim of combating glioma progression and improving patient outcomes.

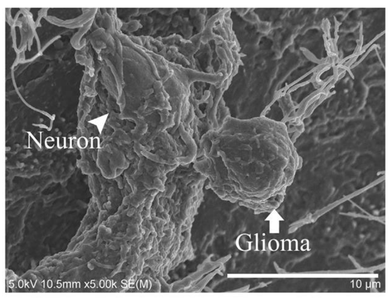

Figure 1. Three dimensional image of perineuronal satellitosis. Diffuse glioma cell (arrow) and neuron (arrowhead) are shown. Taken from Miyai, M. *et al.* (2023).

The main objective of this project is to develop an innovative, reproducible in vitro model that integrates tumor cells and their environment. This model aims to replicate the interactions between the tumor and brain tissue, as well as the invasion process. Furthermore, we seek to compare the behavior of different glioma lineages within this model, including astrocytomas versus oligodendrogliomas and low-grade versus high-grade gliomas.

METHODS

To create this model, we used mini-brains made from mouse embryonic stem cells (ESC) or iPS (induced pluripotent stem) cells. These mini-brains are 3D structures containing neuronal and glial cells, as well as immune cells. They can be maintained for several weeks or even months. In summary, we employed two different models of brain organoids. These organoids were engrafted with DLGG GFP+ cells derived from patient's samples. We studied two different cell lines, from different grades (low and high) and subtypes (astrocytoma and oligodendroglioma).

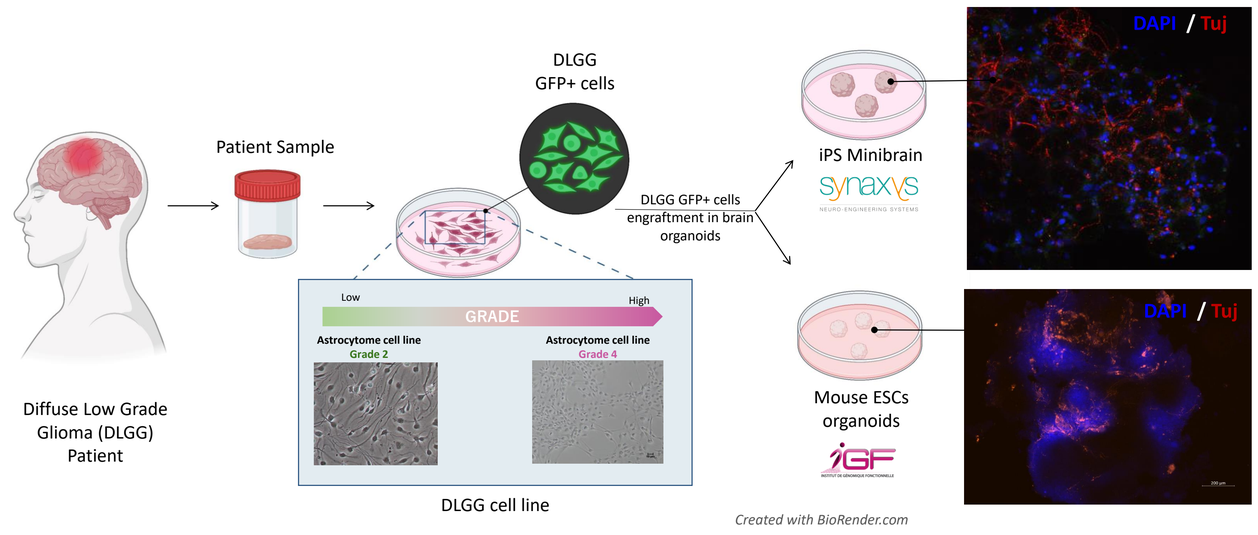

Figure 2. General workflow. Once the DLGG patient undergoes total tumor resection, a sample is obtained from which a cell line is isolated. Two cell lines were used: astrocytoma cell line grade 2 and astrocytoma cell line grade 4, the cell lines were infected to be GFP+ in order to follow them into the brain organoids.

RESULTS

Synaxys Minibrain and LGG cell line integration

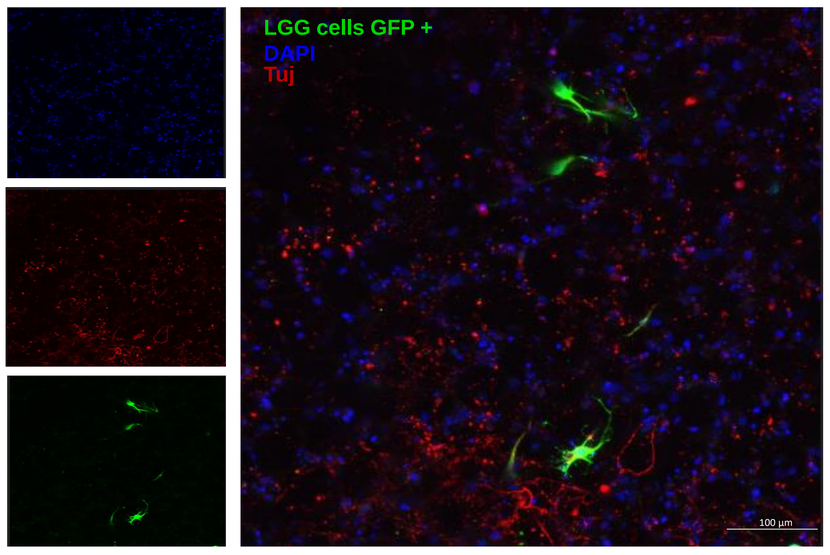

Figure 3. Brain organoids from brain iPS incorporate LGG cell lines.

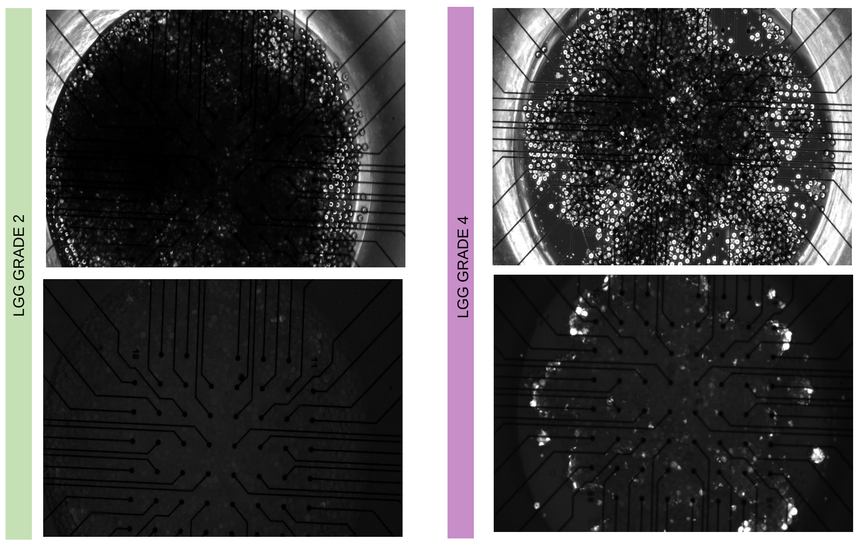

Figure 4. Infiltration of the DLGG cell lines in the brain organoid. Low grade glioma cells (left panel) and high-grade glioma cells (right panel). In vivo epiflorescence monitoring of glioma cell migration through the minibrain.
When culture with the mini-brain model, high-grade glioma cells are more invasive in comparison low grade cells.

Mouse neuronal differentiated cells and LGG cell lines organoid

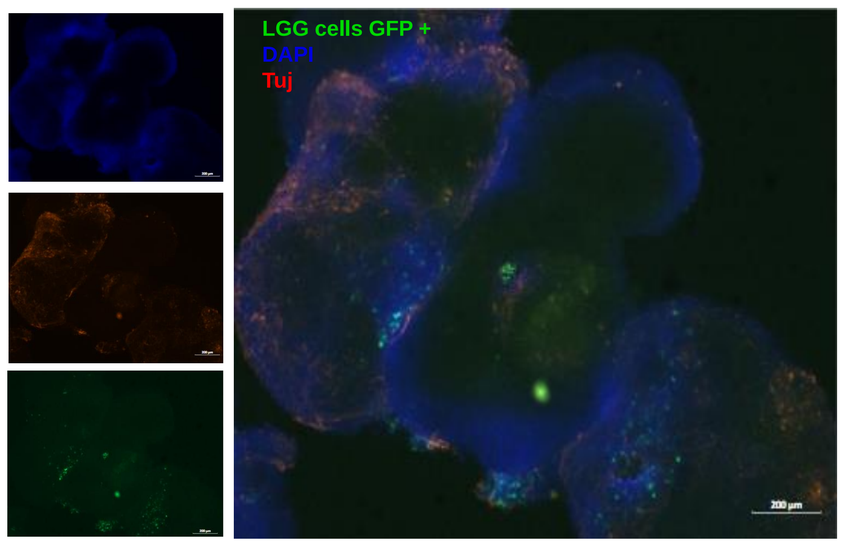

Figure 5. Neuronal differentiated cells and LGG cell lines can form an organoid. Mouse ESCs were differentiated into neuronal precursor cells and culture to form brain organoids, after its formation and co-cultured with the DLLG cell line.

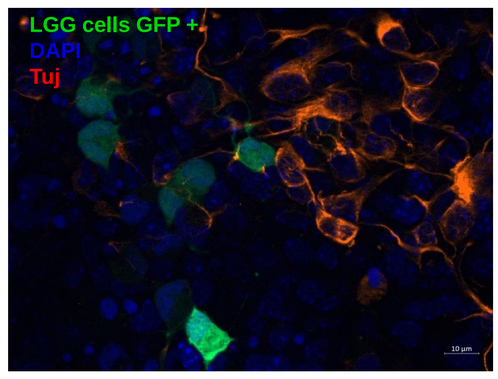

Figure 6. Neuronal differentiated cells and LGG cells integrate and form direct contacts.

NPCs organoids as well as iPS organoids can integrate DLGG cells from patients' resection. Glioma cells infiltrate the organoid and even interact with one another.

The next step in our research entails confirming the results we've obtained. This further validation of our findings will not only support our study but also provide insights into the dynamic interplay between neuron and DLGG cells.

CONCLUSION

In conclusion, the use of models involving the isolation of DLGG cell lines from patients' resection and their placement within brain organoids represents a promising tool for gaining deeper insights into the complex behavior of DLGG.

By gaining a more comprehensive understanding of how different types and grades of DLGG interact and evolve within the organoid, we could be better equipped to design targeted therapies that can address the specific characteristics and challenges posed by these tumors.

This approach will allow us to explore various aspects of DLGG behavior, focusing on key factors such as invasion, self-organization, integration, and tumor heterogeneity within the intricate environment of the organoid.

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