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The Influence of Tumor Microenvironment on the Progression of Glioblastoma

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Abstract:

Glioblastoma (GBM) is a prevalent and highly aggressive primary brain tumor originating from glial cells. The etiology of GBM remains incompletely understood, although certain risk factors (advanced age, exposure to ionizing radiation, and specific genetic mutations) have been identified. TME in GBM is a multifaceted and dynamic system consisting of diverse cell types and extracellular matrix constituents. The TME comprises tumor cells, immune cells (macrophages,T and dendritic cells), stromal cells (fibroblasts, endothelial cells), and soluble factors (cytokines, growth factors). Several unique features characterize the TME in GBM, such as a disrupted blood-brain barrier, hypoxia, acidosis, and a highly immunosuppressive milieu, which collectively contribute to the disease's invasive and aggressive nature and its resistance to conventional therapies like chemotherapy and radiation. Immune cells within the TME can exert pro- or anti-tumor functions, but in GBM, the balance favors a pro-tumor environment. Tumor-infiltrating lymphocytes are scarce in GBM, and their function is often impaired or immunosuppressed. Tumor-associated macrophages (TAMs) are the most abundant immune cell type in the TME, making up to 30% of the tumor mass. TAMs in GBM have a distinctive pro-tumor function characterized by the low production of inflammatory cytokines, the lack of T cell co-stimulation, and the release of immunosuppressive factors. In addition to immune cells, stromal cells and extracellular matrix constituents within the TME can also contribute to tumor growth and invasion. For instance, astrocytes and neurons can provide mitogenic signals to promote tumor growth, while extracellular matrix constituents like hyaluronic acid and tenascin-C can enhance tumor invasion and metastasis. The intricate and dynamic interactions within the TME in GBM pose significant challenges for developing effective therapies. The importance of TME has led to research and engineering of models to allow better understanding of TME, its interaction with the tumor and its effect on tumor progression.

Keywords: tumor cells, extracellular matrix, glioblastoma

• Introduction

Glioblastoma (GBM) is the most common primary brain tumor in adults. It comprises about half of all primary CNS tumors. It has an aggressive nature and a very poor prognosis. Median survival rate is about 15 months after diagnosis, while 5-years survival is only 5%. Complexity of the tumor microenvironment (TME) and sophisticated communication and interaction between TME components and GBM cells, have been identified as major contributors to the poor efficacy of many treatments including immunotherapies. Understanding and manipulating the complex interplay among the different components within TME, are areas of very active cancer research.

This is a preliminary report that outlines a research study of TME of GBM, designed as part of doctoral studies in Cell Biology at UMFT. The study involves engineering a GBM TME model using SynTumor 3D Cancer Model platform and employing the model in a better understanding of the GBM TME, the interaction between the two, as well as its effects on the progression of GBM tumors. Our objective and hope is that a better understanding will allow for a modest contribution to the increase in the knowledge and aid in the development and advancement of more effective treatments and improvement of patient outcomes.

• Materials and Methods

A Glioblastoma Tumor Microenvironment model (GBM-TME model) is engineered using SynTumor 3D Cancer Model platform. Details about SynTumor 3D can be found at https://www.synvivobio.com/syntumor/ GBM-TME model uses patient derived tumor cells and mimics the complexity of TME via careful setup and subsequent tuning of the SynTumor 3D environment.

Things recreated in the engineered model that mimic TME:

1. TME components such as Extracellular Matrix ECM, Dendritic Cells DC, Glioma Stem Cells GSC, Macrophages,etc.
2. 3D Architecture to facilitate interaction and communication between components.
3. Mechanical properties such as density and volume.
4. Blood Brain Barrier.
5. Angiogenesis & hypoxia.
6. Leaky vasculature.

GBM-TME model is employed in a research study for better understanding of the TME, its evolution over time, the interplay between TME and GBM cells, as well as tumor progression.

The study involves the following steps:

1. Develop 3D Cultures of primary (de novo) glioblastoma cells on SynTumor 3D to create cancer models with physiologically and morphologically realistic tumor microenvironments (TME).
2. Develop 3D Culture of normal brain tissue on SynTumor 3D to be used as a control model.
3. Investigate the composition of TME, by identifying/measuring main TME components and studying the evolution of TME composition over time.
4. Compare the composition of TME with the composition of the microenvironment of the normal brain tissue model.
5. Study the interplay/dynamics between the tumor and TME, as reflected by the presence of signaling pathways and communication mechanisms/elements
6. Study TME components that are contributing to immunosuppression.
7. Study tumor progression over time in conjunction with the evolution of TME composition and presence of immunosuppressive factors.
8. Experiment and research the implications of the TME on immunotherapies.
9. Study the efficacy of current treatments on 3D Cultures with different TMEs and identify TME components and immunosuppressing factors that contribute to reduction in efficacy.

• Results and Discussion

While still in its preliminary phase, the research study should be able to confirm the following:

- TME suffers substantial evolution and changes over time and in different microregions.
- Glioma Stem Cells GSC and TME perform cooperative signaling, which promotes neovascularization and maintenance of GSC. In addition, GSCs are supporting elements in neovascularization by secreting angiogenic factors such as Vascular Endothelial Growth Factor VEGF.
- Leaky vasculature allows access to nutrients for GBM and TME components.
- There are endothelium activated pathways that promote GSC self-renewal and therapy resistance.
- GSCs resist both chemotherapy and radiotherapy, so they should be candidates for targeted adjuvant therapies.
- Radiotherapy triggers remodeling of TME, which in turn contributes to tumor cell survival during radiation treatment.
- Standard chemotherapy with temozolomide (TMZ) involves TME remodeling too; this promotes a resistant, pro invasive tumor phenotype.
- GBM cells invade along anatomical tracks, such as perivascular space or myelinated axons.
- Presence of Hyaluronic Acid in TME drives tumor progression.
- Presence of stromal cells has a strong impact on GBM cell behavior; for example, GBM cell cultures with astrocytes and endothelial show higher migration speed and resistance when compared to GBM cell culture alone.
- Spatial variation in mechanics and TME composition influence tumor progression.

The research study still faces some challenges related to:

- accuracy of the model to make it better predictive of in vivo behavior. This requires tuning of a number of physical parameters of the model, to better match those of the brain (ex. TME composition and mechanics).
- inclusion of patient derived tumor cells.

These challenges will be addressed as the study goes along.

• Conclusions and Further Research

The research study demonstrates the complexity of TME and the sophisticated communication and interplay between TME and GBM tumor. GBM-TME communication and interplay promote tumor survival and its progression Considering its strong influence on tumor progression, TME is a valid therapeutic target for GBM treatment, as well as warrants further research. By incorporating specific TME attributes (composition, mechanics, topography, stromal cells, etc.) engineered 3D microenvironments such as GBM-TME model are critical tools in this research.

At the same time, further studies can include the development of Organoid Models of Growth and Invasion, as well as 3D Bioprinting.

Organoid Models of Growth and Invasion involve seeding patient tumor samples in 3D matrices and stimulating them to develop naturally into organoids. The organoids would be a much better reflection of the evolution of GBM tumor in vivo and the GBM-TME dynamics that facilitate this evolution.

3D Bioprinting involves 3D printing of resected GBM tumors from real patients in order to fabricate 3D matrices which are accurate GBM-TME models of those tumors and are able to recapitulate clinically observed patterns of tumor resistance to standard therapeutic treatments of those patients.

Over time, the research employing these models will offer the opportunity to rapidly and precisely dissect mechanisms of GBM progression, accelerate clinical testing and provide a platform for precision medicine.