Necrotic Core-Mediated Immune Suppression in a Patient-Derived Glioblastoma-on-Chip Model

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Abstract

Necrosis emerges as a key driver of immune suppression in glioblastoma. Using a patient-derived organ-on-chip model, we demonstrate that necrotic niches induce T cell exhaustion and macrophage polarization, reshaping the immune landscape. Our findings uncover necrosis as a potential target to improve immune responses and guide new immunotherapy strategies for glioblastoma.

Introduction

Glioblastoma (GBM) is the most lethal primary brain tumor in adults, characterized by infiltrative growth, profound heterogeneity, and resistance to current therapies (1). Despite multimodal treatment, survival outcomes remain dismal, with a median overall survival of approximately 15 months (2). The failure of immunotherapies in GBM is largely attributed to its immunologically "cold" tumor microenvironment (TME), marked by poor T cell infiltration and dominance of immunosuppressive myeloid cells (3).

A key contributor to this immunosuppressive environment is necrosis—a pathological hallmark of GBM—yet its specific impact on immune function remains incompletely understood. Necrotic areas, which develop under hypoxic and nutrient-deprived conditions, release damage-associated molecular patterns, cytokines, and metabolites capable of reshaping immune cell behavior (4). However, conventional 2D models fail to recapitulate the spatial complexity and hypoxic gradients of the GBM TME (5,6). To address this gap, we developed a human organ-on-chip (OOC) model that recreates the necrotic core and its surrounding microenvironment. Through integration of single-cell RNA sequencing (scRNA-seq) and flow cytometry, we dissected how necrosis reprograms immune cell function, uncovering exhaustion markers and immunosuppressive shifts that could inform next-generation immunotherapy approaches.

Materials and Methods

Primary GBM cell lines were established from surgical resections provided by the Biobank of the Aragon Health System. PBMCs were isolated from healthy donor buffy coats (Banco de Sangre y Tejidos de Aragón).

A collagen-based organ-on-chip system was used to model the GBM microenvironment. Necrosis was induced by pre-culturing tumor cells for 5 days before adding PBMCs; control chips received immune cells immediately after tumor cell seeding.

After 48 hours of co-culture, confocal microscopy was used to assess viability, hypoxia, and immune infiltration. Supernatants were analyzed by ELISA for IL-12, TNF-α, and TGF-β. Tumor and immune cells were collected for flow cytometry and gene expression analysis using RT² Profiler PCR Arrays. $CD45^+$ cells were further profiled by single-cell RNA sequencing (10x Genomics).

Results and Discussion

Using patient-derived GBM cells cultured in our OOC platform, we successfully recapitulated key features of the TME, including the formation of necrotic cores driven by hypoxia gradients. Time-lapse imaging and flow cytometry revealed a

progressive loss of cell viability within the tumor core, with significant increases in hypoxia and necrosis-linked cell death. This hypoxic core impaired immune cell infiltration, reduced cell motility and killing efficiency, and altered overall immune dynamics. Notably, co-culture experiments with PBMCs showed that immune cells actively targeted and killed GBM cells under normoxic conditions, but this response was strongly diminished in the presence of a necrotic core.

Single-cell RNA-seq and flow cytometry further confirmed that necrosis reshaped the immune landscape, inducing loss of effector monocytes and $CD4^+$ T cells, and expansion of Tregs and M2-like TAMs. A unique immune subpopulation associated with improved patient survival was completely lost under necrotic conditions. This necrosis-driven immunosuppressive reprogramming was reinforced by elevated expression of exhaustion markers in $CD8^+$ T cells, validated by both transcriptomic and flow cytometry data. Interestingly, NK cell subsets under necrotic conditions showed dual activation/inhibition signatures, suggesting functional plasticity that might be therapeutically targetable.

Conclusions

Our findings highlight necrosis not merely as a histological hallmark, but as a dynamic, immunomodulatory driver of immune evasion in glioblastoma. These insights underscore the importance of targeting necrosis-associated pathways to reverse immune suppression and enhance the efficacy of immunotherapies in GBM.

Our organ-on-chip model offers a high-fidelity platform for further exploration of combinatorial treatments that restore immune competence in the necrotic tumor microenvironment.

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