Effect of Glioblastoma Tumour Microenvironment on the Modulation of the Immune System

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Abstract

Glioblastoma (GBM) is the most common and lethal malignant primary brain tumour in adults. Understanding its function and microenvironment is crucial to develop effective treatments. In this study, we optimized the co-culture of glioblastoma cells with immune cells by measuring cytokine secretion with interleukin-2 stimulation in different environments.

Introduction

Influence of glioblastoma microenvironment on immune surveillance.

Glioblastoma, also known as grade 4 astrocytoma, is a highly infiltrative and rapidly progressive type of tumour and is therefore considered to be the most lethal primary brain tumour in adults. Patients suffering from this type of tumour are treated with the standard of care, based on radiotherapy and chemotherapy, with concomitant and adjuvant temozolomide after surgical resection [1]. Despite this, the average survival of patients remains very low; about 14 months. Tumour microenvironment (TME) is closely linked to the immunogenic capacity of the tumour itself, thus establishing low/no immunogenic tumours (cold tumours) and immunogenic tumours (hot tumours) [2]. Glioblastoma TME is characterised by being highly immunosuppressive and having low immunogenicity, highlighting it as a 'cold tumour'. This immunosuppressive environment is due to several factors, both cellular and non-cellular. On the other hand, the migration of immune system cells and access to tumour antigens is an aspect of utmost importance for the induction of antitumor immunity. The effective functioning of immune system cells is suppressed through various mechanisms, and one of them is the secretion of diverse cytokines. Cytokines are secreted proteins that engage the extracellular domains of cell surface receptors and regulate immune response and homeostasis. They can be classified based on their role as pro-inflammatory related cytokines (e.g., TNF-α, IL-12) or anti-inflammatory cytokines (TGF-β) [3]. There is communication between glioma cells and immune cells through TGF-β, which, among other functions, is responsible for reducing the cytotoxic activity. Glioma cells secrete TGF-β, that participates in M2 polarization of macrophages, generating an immunosuppressive environment, as they will in turn secrete more of this cytokine, reducing the cytotoxic capacity of immune cells in the microenvironment. This is due to a tumour-induced decrease of cytotoxic T cells and pro-inflammatory cytokines (IL-2, IFN-γ, IL-12 and TNF-α), and at the same time, an induction of immunosuppressive cytokines (TGF-β) and immunosuppressive cells such as tumour-associated macrophages (TAMs) [4]. Therefore, glioblastoma can modulate its immune microenvironment into an immunologically cold tumour.

Our Approach

Our approach consists of generating different co-culture conditions to recreate a physiological environment similar to *in vivo* conditions. To carry out a co-culture it is very important to find the optimal conditions for all populations. The co-culture of U-251 MG cell line derived from Glioblastoma, transfected with the green fluorescent protein (GFP) and human peripheral blood mononuclear cells (PBMCs) was performed in two-dimensional cell culture models. To optimize the conditions, both populations were seeded together and separately in 24-well plates and different factors were studied, such as the type of medium in which they are cultured, the effect of cryogenic stress, the stimulation of PBMCs by IL-2 and the effect that the populations have on each other. To observe which conditions were the most optimal, viability tests were

performed with propidium iodide (PI) and calcein AM (CAM) stains.

On the other hand, after optimization with different culture conditions, a study of cytokine secretion by the different populations in the culture conditions that were considered more physiological was also carried out. For this purpose, supernatants from the selected conditions were collected and immunoassay ELISA was performed to observe the cytokines secreted. We studied the secretion of the cytokines IL-12 and TNF-α, which are more related to antitumor responses, and the secretion of TGF-B, which is more related to the protumour or immunosuppressive response.

Conclusions

The tumour microenvironment has a complex influence and interaction with the immune system cells that access it. Being able to know what are the conditions that occur in a tumour *in vivo*, allows us to represent in a faithful way in our models. In this study, we were dedicated to optimize co-culture conditions regarding culture time, stimulation of immune cells or stress conditions of immune cells. Furthermore, in our work, we have been able to identify the secretion of cytokines by both U-251 MG cells and PBMCs. This cytokine analysis has also allowed us to understand the intercellular interaction within the TME and the signalling battle that takes place during immune surveillance. Our next step is to apply the optimized settings into an organ-on-chip cell culture model, that represent the real physiology of the tumour, which is essential for effective research.

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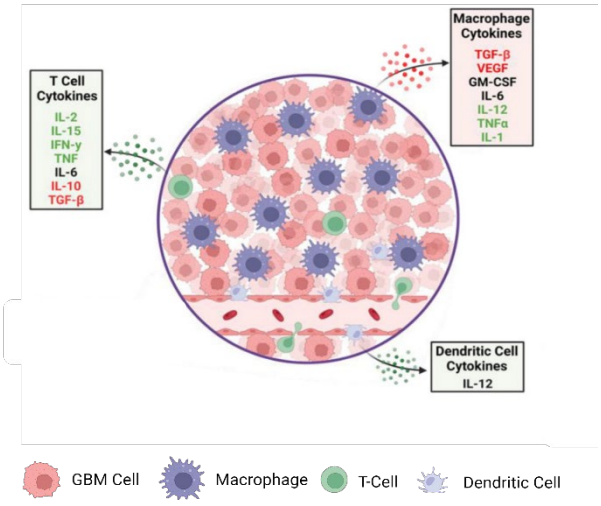

Figure 1. Intercellular interaction and associated cytokine dynamics. Adapted from SOORESHJANI M. et al., (2023).

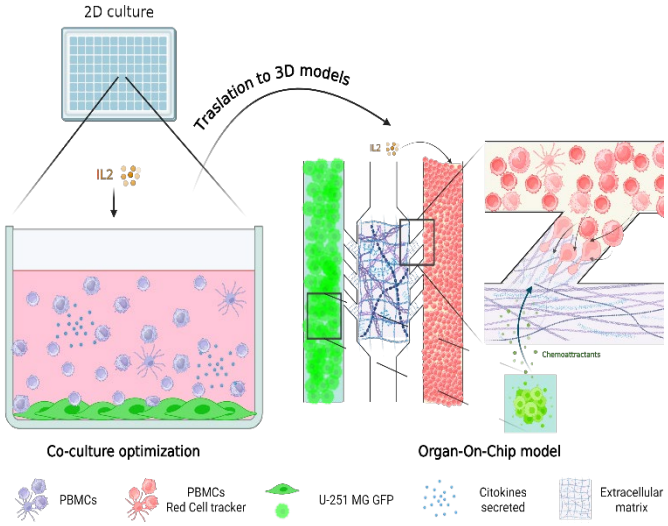

Figure 2. Graphical representation of three-dimensional co-culture optimization