Impact of tumour microenvironment stiffness on immune cell behaviour using Organ-on-chip models

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Abstract

The extracellular matrix (ECM) is one of the main factors that are modified by glioblastoma. The tumour is able to increase the stiffness of the ECM, hindering the infiltration of the immune system into the tumour. Simulating this whole network is a difficult task to accomplish in conventional cellular models. Therefore, this study uses organ-on-chip devices to simulate the tumour microenvironment and study the effect of ECM stiffness on immune cell behaviour.

Introduction

Glioblastoma and its microenvironment

Glioblastoma (GBM) is the most common malignant brain tumour in adults, characterised by a rapid growth and a poor prognosis. Despite current treatment, consisting of a combination of surgery (when possible), radiotherapy and chemotherapy with Temozolomide [1], tumour recurrence is inevitable. One of the major factors contributing to poor survival is the great heterogeneity of the tissue microenvironment (TME), which hinders efforts to study how the GBM actually works and how it interacts with its environment, mostly constituted by immune cells and extracellular matrix. The ECM forms the acellular part of a tissue, a meshwork of components such as collagen, laminin and hyaluronan (HA), among many others, that provide structure and support to the surrounding cells. The ECM, however, plays a much more important role in a tumour such as GBM. Many components of the ECM, such as collagen, have been found to be overexpressed, making the matrix much stiffer and denser than in normal brain tissue [2]. This limits, for example, the ability of some chemotherapeutic agents to penetrate the tumour, thereby reducing their effectiveness and worsening the prognosis of patients. In addition, these matrix components also mediate the immune response. ECM stiffness may inhibit the infiltration and migration of immune cells such as T lymphocytes, contributing to a suppression of immune surveillance against the tumour. In human and mouse GBM, it has been shown in vivo that tumour areas with a higher abundance of ECM fibres had a lower infiltration of T-lymphocytes. However, few in vitro models have been able to replicate these conditions [3]. In addition to this process of immune cell infiltration, there is another prior mechanism. Under the effect of a variety of pathophysiological conditions, immune cells cross from the bloodstream into the target tumour tissue through the endothelial barrier of the blood vessel in which they are located. We now understand that this process, known as extravasation, is produced by a close communication between the surface of the immune cells and the endothelial barrier, where a myriad of adhesion molecules, intracellular signals and chemotaxis participate to activate the infiltration capacity of the immune cells.

Organ-on-chip technology

A complex mechanism occurs from the moment an immune cell receives a signal to attach to the endothelial wall and pass through it, until it infiltrates the tumour tissue [4]. In addition to all the above, the great complexity and heterogeneity of the tumour microenvironment makes the study of tumour behaviour a very difficult task with conventional culture models. The lack of preclinical models capable of mimicking the complexity of the GBM makes it difficult to obtain answers and treatments on tumour-immune system and tumour-ECM interactions. In this context, organ-on-chip technology represents a paradigm shift by allowing the recreation of microenvironments very similar to *in vivo* tumour conditions, bringing us one step closer to deciphering the intrinsic mechanisms that promote GBM progression.

Our approach

Our approach consists of a microfluidic device containing two side channels separated by a central chamber where the extracellular matrix is incorporated. The major advantage is the absence of a physical barrier between the chamber and the channels, allowing direct interaction of the different cell types with the matrix. U-251 MG cells, derived from GBM, are seeded in one of the side channels, which secrete a series of chemoattractants that produce a call effect and activate immune cells, located in the other side channel. In addition, our model allows modification of the extracellular matrix stiffness present in the central chamber. It has already been shown that stiffness has a strong influence on tumour progression. Therefore, we are going to study the behaviour of immune and GBM cells in a 3D microenvironment: how they infiltrate the ECM, what genotypic changes on stiffness induces, etc.

The first thing we observed was an insufficient activation of immune cells in the presence of normal culture medium. However, after adding interleukin-2 (IL-2), the cells activated, proliferated and infiltrated the ECM. This interleukin is normally produced by immune cells when exposed to a foreign agent, but we found that there was insufficient stimulus for immune cell activation, and that the addition of this molecule significantly enhances immune activation and infiltration. Then, the effect of matrix stiffness on the behaviour of immune cells in response to GBM signalling was studied. For this purpose, several conditions were performed. Microfluidic devices were seeded with two concentrations of collagen (2 and 4 mg/mL) in the central chamber, U-251 MG cells in one side channel, and immune cells in the other. Additionally, endothelial cells (HBEC) were seeded at the interface between the collagen matrix and the immune cells, acting as the endothelial barrier of a blood vessel. Finally, a control was performed on microfluidic devices without U-251 MG cells in the side channel. With this, 12h videos were recorded to follow the immune infiltration in the ECM under different environments.

The videos showed that the immune cells rapidly infiltrated and invaded the low-density matrix, while they failed to enter any of the devices with a high-stiffness matrix, although the cells remained active. However, incorporation of the endothelial barrier into high-stifness ECM devices appeared to induce adhesion of immune cells and they were able to cross the rigid matrix interface. On the other hand, controls did not induce infiltration, and even the absence of tumour stimuli resulted in cell death due to lack of activation.

Conclusions

The extracellular matrix and modifications in its stiffness by the GBM modulate the immune infiltration and its action against the tumour. The study of the tumour microenvironment, composed of tumour cells, immune cells and the extracellular component, is a very complicated task in conventional cell culture models. In this study, we simulate the mechanobiological characteristics of the TME in organ-on-chip devices, as well as key mechanisms in the process of the immune response against the tumour. With this, we will be able to study the infiltration, proliferation, exhaustion and adhesion of immune cells, as well as their direct interaction with a complex GBM microenvironment. A better understanding of mechanisms leading to immune suppression could help the development of more effective therapies in this aggressive tumour.

REFERENCES

[1] STUPP, R. *et al.* Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. *New England Journal of Medicine*. 2005, doi:10.1056/NEJMoa043330.

[2] KUCZEK, D.E. *et al.* Collagen density regulates the activity of tumor-infiltrating T cells. *J Immunother Cancer*. 2019, doi:10.1186/s40425-019-0556-6.

[3] MOHIUDDIN, E. AND WAKIMOTO, H. Extracellular matrix in glioblastoma: opportunities for emerging therapeutic approaches. *Am J Cancer Res*. 2021, pp. 3742–3754.

[4] J. D. VAN BUUL AND P. L. HORDIJK. Signaling in Leukocyte Transendothelial Migration. *ATVB*. 2004, doi:10.1161/01.ATV.0000122854.76267.5c.

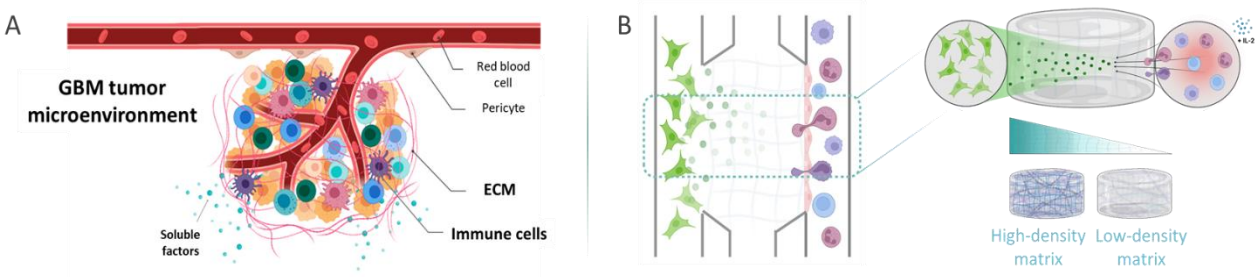

Fig. 1. (A) GBM tumour microenvironment and its components. (B) Our Organ-on-chip device with GBM cells (green) in the left and immune cells in the right channel.