Chapter 1

Introduction

1.1 Background

Glioblastoma (GBM) is a World Health Organization (WHO)-ranked Grade IV cancer that arises when glial cells within the brain undergo uncontrollable proliferation due to genetic mutations (Hanif et al., 2017; Kanderi, 2024). Its high ranking of danger was given due to the extremely aggressive and invasive nature of GBM tumor cells, which generally grants patients an average survival time of only 12–15 months and a recurrence rate of up to 90% (Minniti et al., 2021; van Linde et al., 2017). Unfortunately, GBM is also the most prevalent type of glioma or central nervous system (CNS)-related tumor, representing nearly half of all its cases and occurring in 3–5 people in a population of 100000 (Alifieris & Trafalis, 2015; Grech et al., 2020; Philips et al., 2018).

Due to interests in preserving crucial brain function, conventional cancer treatments such as chemotherapy, radiotherapy, and tissue removal are typically regarded to be highly risky, thus the need for the development of a safer therapeutic approach. Like in other cancers, immunotherapy has also been regarded as a prospective treatment option for GBM, due to the ability of immune cells to travel to targets with ease, accurately detect tumor cells, and retain memory to minimize the chances of recurrence (Chu et al., 2022). However, the development of GBM-specific immunotherapy has been significantly delayed due to the uniquely-immunosuppressive nature of the GBM tumor microenvironment (TME), which can downregulate the production of the anti-tumor immune cells or chemical signals (Pinheiro et al., 2023).

Attempts to further track the trigger of immunosuppression in GBM has led to suspicions of macrophages being heavily involved. Macrophages, which typically mediate cytotoxic properties

towards foreign pathogens and cancer cells, may be polarized into pro-tumor variants known as M2 macrophages when exposed to certain chemical signals like interleukin (IL)-4, IL-10, and IL-13, many of which have been found to be present in the GBM TME (Khan et al., 2023). Among its many pro-tumor actions, M2 macrophages have been found to secrete platelet-derived growth factor (PDGF), which can support GBM survival upon binding to platelet-derived growth factor receptor (PDGFR) on its surface (Pandey et al., 2023). Furthermore, a recent breast cancer study by Kim et al. (2023) have found links between the proteins platelet-derived growth factor receptor alpha (PDGFRA), signal transducer and activator of transcription 3 (STAT3), and IL-6. IL-6, in particular, is a known immunosuppressive cytokine that can strongly inhibit the production or entry of anti-tumor immune cells, such as T-cells and natural killer cells, into the TME, thus allowing cancer progression to continue undisturbed (Rašková et al., 2022). These new findings have brought forward the possibility that confirming the connection between M2-mediated PDGFR, STAT3, and IL-6 signaling may provide insight into resolving the poor efficacy of immunotherapy against GBM.

1.2 Objectives

To investigate the effect of macrophage-induced PDGFR signaling on IL-6 production in GBM, three experiments were performed to tackle three objectives, namely:

- To confirm the presence of macrophage and IL-6 within the GBM TME with the immunofluorescence staining of IL-6 and the macrophage marker F4/80 on CT-2A GBM-injected mouse brain tissue models.
- To compare the expressions of key proteins in the proposed PDGFR/STAT3/IL-6 pathway in PDGFR-activated A172 GBM cells cultured with macrophage cells and isolated PDGF, as well as PDGFR-inhibited A172 GBM cells, using western blotting.
- To gain insight for future research related to the possible PDGFRA/STAT3/IL-6 pathway by assessing the correlations between PDGFs, PDGFRs, STAT3, and IL-6 RNA transcription levels in GBM patients from existing databases.

It was hoped that this research may greatly contribute to the complete mapping of the numerous complex interactions that occur within the GBM microenvironment to further advance the development of effective treatments for this highly-devastating illness.

1.3 Hypotheses

Based on the findings of Kim et al. (2023), it was hypothesized that macrophage-induced PDGFRA activation would also increase IL-6 expression in A172 GBM cells. Thus, the formulated hypotheses for experiments done may be seen below:

Immunofluorescence staining	In Vitro Protein Analysis	In Silico Correlation Analysis
H_0: F4/80 and IL-6 fluorescence will be **brighter in the non-tumor** area of the CT-2A GBM-injected mouse tissue section	H_0: PDGFR inhibition **will not significantly decrease** IL-6 expression in A172 GBM co-cultured with THP-1 macrophage cells	H_0: There is **no significant correlation** among the RNA transcription levels of PDGFs, PDGFRs, STAT3, and IL-6 in GBM patients
H_1: F4/80 and IL-6 fluorescence will be **brighter in the tumor** area of the CT-2A GBM-injected mouse tissue section	H_1: PDGFR inhibition **will significantly decrease** IL-6 expression in A172 GBM co-cultured with THP-1 macrophage cells	H_1: There are **significant correlations** among the RNA transcription levels of PDGFs, PDGFRs, STAT3, and IL-6 in GBM patients