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IGF2BP1 in Mesenchymal GBM Immune Signalling Regulation via c-Myc

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Abstract: Glioblastoma multiforme (GBM) is a highly aggressive and lethal brain tumor, with poor patient prognosis and median overall survival of only 10 months despite the current Stupp protocol treatment, due to its high aggressiveness and recurrence rate. This study investigated the role of the IGF2BP1 gene in mesenchymal glioblastoma multiforme (GBM), revealing that IGF2BP1 is upregulated in tumor tissues compared to adjacent normal tissues and positively correlates with MYC gene expression and poor patient prognosis. Immune infiltration analysis showed that IGF2BP1 is associated with specific immune cell populations, and GSVA analysis confirmed its positive correlation with the immune functions of most B cells and macrophages. The mechanism of IGF2BP1 regulating c-Myc expression in mesenchymal GBM and its subsequent impact on immune-related signalling pathways, thereby affecting the immune microenvironment of tumors and patient prognosis, provides new targets and ideas for future immunotherapy of mesenchymal GBM.

Keywords: Glioblastoma multiforme; Immune; IGF2BP1; c-Myc

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1. Introduction

Glioma primarily originates from glial cells within the brain represent the most common type of tumor in the central nervous system (CNS), accounting for 30% of all primary brain tumors and 80% of all malignant brain tumors [1]. According to the World Health Organization (WHO) classification, grade 4 glioma, known as glioblastoma (GBM), comprises approximately 57% of all gliomas, with an average annual incidence rate of 4.23 per 100,000 individuals [2]. GBM is characterized by its highly aggressive nature and a markedly high mortality rate, evidenced by a 5-year survival rate of merely 6.8% post-diagnosis. Its development is associated with various factors, including environmental influences, genetic variations, and inherited syndromes [1–3].

Currently, the Stupp standard plan is a widely adopted clinical treatment plan for GBM, which is to surgically remove the tumor within the maximum safety range, followed by temozolomide concurrent chemotherapy and radiotherapy, and then temozolomide maintenance chemotherapy starting four weeks after the end of

radiotherapy. However, the prognosis for patients remains poor, with a median overall survival (OS) of merely 10 months [4–7]. Additionally, targeted treatment methods based on specific genetic mutations or abnormalities in signaling pathways, including the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) and the p53, retinoblastoma (RB), and epidermal growth factor receptor (EGFR) pathways [8]. The failure of targeted drugs in the later stages of clinical development suggests that most GBM multiforme is not even close to being a single-pathway-driven disease that can be treated with targeted therapy. In addition, the latest immunotherapy chimeric antigen receptor (CAR)-T cell therapy has not demonstrated sustained antitumor activity against GBM multiforme [9]. In addition to the limitations of CAR-T cells in their ability to migrate and infiltrate solid tumors, the antitumor cytotoxicity of CAR-T cells is also constrained by the immunosuppressive TME containing immunosuppressive cells and immune checkpoint molecules that promote immune escape [10]. Therefore, studying the interaction between GBM tumor cells and immune cells, especially exploring the mechanisms by which tumor cells secrete factors that actively promote tumor cell proliferation and invasion and act on immune cells to form a tumor immunosuppressive microenvironment in the TME, can guide the development of new immune interventions and improve the prognosis of patients with GBM.

GBM microenvironment encompasses vascular compartments and immune cells such as monocytes, macrophages, microglial compartments, or T cells [8,11,12]. GBM promotes tumor growth, proliferation, and invasion by altering the interaction of immune cells and modulating the mutual regulation of immune molecules, thereby influencing both innate and acquired immunity through mechanisms of immune evasion. It has been demonstrated that the depletion of glioma-associated microglia/macrophages (GAMs) can significantly reduce tumor growth [13,14]. Compared with traditional treatment, immunotherapy, such as immune checkpoint inhibitors and tumor vaccines-targets specific tumor cells without damaging normal cells, potentially reducing side effects [15–17]. Moreover, by activating the patient's immune system to combat the tumor, immunotherapy can provide more durable effects, continuously monitoring and eliminating residual tumor cells to prevent recurrence [16,17]. However, the efficacy of these treatments varies among GBM patients due to differences in individual conditions, tumor types, immune statuses, and other factors.

In the innate immune response, GBM cells release cytokines such as TGF-β, IL-10, IL-4, and IL-13, promoting the acquisition of an M2 macrophage-like phenotype by microglia, associated with increased invasiveness and poor prognosis [18]. Additionally, GBM stem cells (GSC) can recruit tumor-associated macrophages (TAM) by secreting cytokines related to selective activation like osteopontin, IL-6, IL-8, and TNF-α. TAM further enhances the growth, invasion, and angiogenesis of GBM by secreting various growth factors and inflammatory factors such as TGF-β1, VEGF, epidermal growth factor (EGF), and IL-10 [19–22]. Furthermore, the overexpression of oncogenes and loss of tumor suppressor genes can diminish the sensitivity of cells to immune-mediated killing, facilitating immune evasion in GBM [23].

GBM cells can suppress the cellular immune activity of the adaptive immune system within the GBM microenvironment. GBM-associated myeloid-derived suppressor cells (MDSCs) have the potential to promote the suppressive activity of regulatory B (Bregs) cells towards CD8 T cell activation and acquisition of effector phenotypes by delivering membrane-bound PD-L1-containing microvesicles [24]. Additionally, the TGF-β released by GBM cells can suppress inflammation and promote immunosuppression by inhibiting T cell proliferation and inducing T cell apoptosis [25,26]. Meanwhile, GBM cells can release cytokines such as IL-10, which participate in the upregulation of signal transducer and activator of transcription 3 (STAT3), further suppressing the activity of immune cells [26].

There are differences in the prognosis and survival rates of patients with different GBM subtypes. Based on differences in gene expression, GBM is classified into four subtypes: classical, mesenchymal, neural, and proneural. The mesenchymal subtype, characterized by its high invasiveness and angiogenesis, poses significant challenges for traditional treatment methods, leading to poor patient prognosis due to high recurrence rates and short survival times [27,28]. Additionally, this subtype possesses resistance to apoptosis, further increasing its malignancy and treatment difficulty [29]. Due to the high invasiveness and angiogenesis of mesenchymal GBM, traditional surgical resection, radiation therapy, and chemotherapy often find it difficult to eliminate the tumor. Even with intensive treatment, the prognosis for patients is relatively poor, with a high recurrence rate and short survival time [30]. Compared to the other three subtypes, mesenchymal GBM has the strongest immunogenicity [31]. It not only exhibits a high ratio of macrophages/microglia and an abundance of neutrophils, but also a low abundance of NK cells [30]. Additionally, it can activate M2-type macrophages and suppress T cell activity, thereby mediating immune suppressive TAM [32]. Increased mRNA expression of various cytokines, immune cell markers, and immune-related signaling pathways makes this subtype the most immunologically and inflammatory-associated subtype [33,34]. The immune evasion characteristics of mesenchymal GBM suggest that immunotherapy may be more effective in stimulating immune responses, thereby contributing to the elimination of tumor cells and improving patient prognosis.

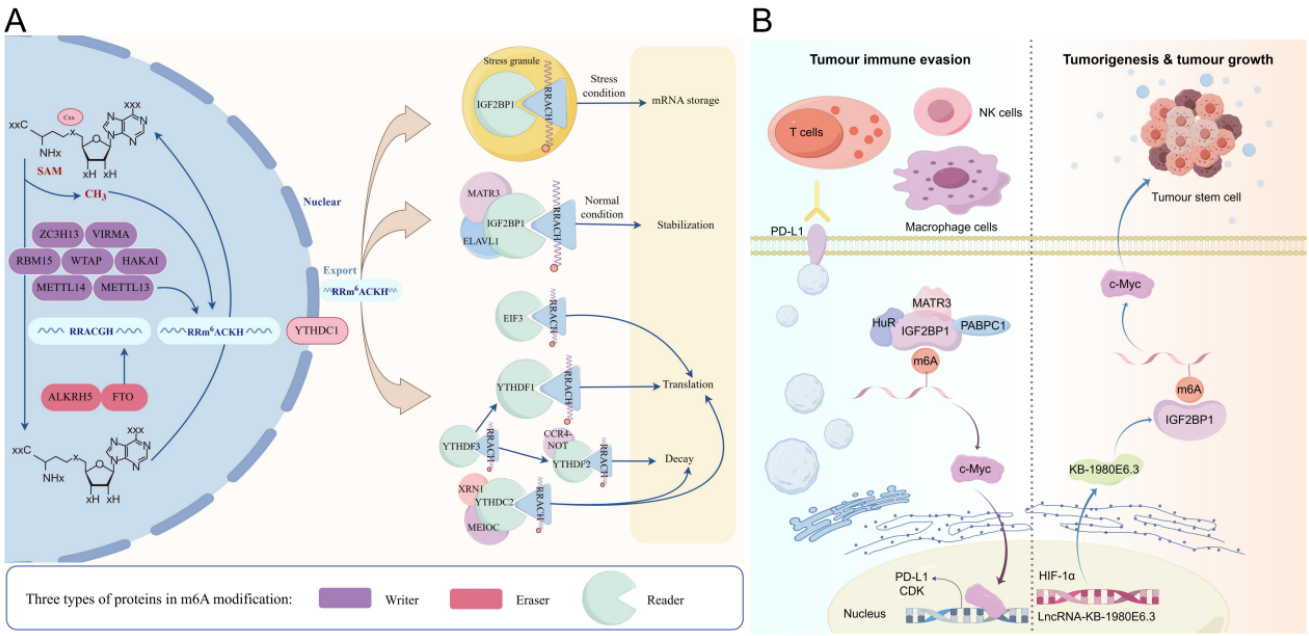

Figure 1. (A) The three classes of proteins—Writers, Erasers, and Readers—dynamically regulate m6A modification flexibly. (B) IGF2BP1 affects the TME and the tumor itself by regulating the expression of c-Myc in other tumors.

As the most abundant modification in eukaryotic mRNAs, m6A is considered the most common, frequent, and conserved internal modification in cancer development [35]. This dynamic regulation is facilitated by three classes of proteins: "writers," "erasers," and "readers" (**Figure 1A**) [36]. "Writer" proteins, such as METTL3 and METTL14, collaborate with the RNA-binding protein WTAP to add m6A modifications to mRNAs [37]. "Eraser" proteins, including FTO and ALKBH5, remove m6A modifications, affecting mRNA stability and translation efficiency [36]. "Readers" such as YTHDF1/2/3, YTHDC2, and IGF2BP1/2/3, recognize and bind to m6A-modified transcripts, influencing gene expression through processes such as mRNA stability, mRNA splicing, and

translation efficiency [36]. Studying the role of m6A RNA modification in GBM has revealed that this modification affects immune responses by regulating the expression of specific genes and intercellular interactions, with reading proteins such as IGF2BP1 playing a crucial role in GBM [38–42]. The upregulation of this protein can regulate the proliferation and survival of GBM cells, modulate the stability and expression of specific mRNAs such as c-Myc, significantly reduce the expression of PD-L1 in the tumor, and consequently promote immune evasion (Immune cells such as CD4, CD8 T cells, CD56 NK cells, and F4/80 macrophages), ultimately leading to tumor progression [38–41]. As a key regulator in the TME, c-Myc promotes the expression of CD47 and PD-L1 by binding to their gene promoters, with PD-L1 expressed on cells such as T cells, B cells, and macrophages, thereby not only inducing exhaustion and apoptosis in CD8 T cells but also suppressing their proliferation and cytotoxicity through binding to PD-1 on their surface [42,43] (**Figure 1B**). Additionally, the upregulation of c-Myc expression not only triggers the expression of cytokines such as IL-4, IL-10, and TGF-β, resulting in the polarization of macrophages towards the M2 phenotype [44], but also prompts B cells to facilitate T cell apoptosis via the expression of PD-L1 and IL-10, and secrete TGF-β to impede the function of NK cells [45], thereby further contributing to immune evasion. Research has found that this gene can also maintain the stem cell properties of tumors and directly promote tumor growth through the LncRNA KB-1980E6.3/IGF2BP1/c-Myc pathway [42] (**Figure 1B**). The immunosuppressive TME formed by various factors, as well as their direct impact on the tumor, lead to the occurrence, migration, and metastasis of the tumor [40–42,46,47].

2. Materials and methods

In this study, a comprehensive approach was employed to investigate the role of the IGF2BP1 gene in mesenchymal glioblastoma (GBM) by utilizing R (2022.02.3 Build 492) and TIMER2.0 (http://timer.cistrome.org/) to analyze gene expression differences between tumor and adjacent normal tissues in GBM from TCGA, conducting statistical tests to compare patient characteristics and outcomes, employing the CIBERSORT algorithm for immune infiltration analysis, performing gene set variation analysis (GSVA) to assess immune process enrichment, and conducting cell culture and coculture experiments using a Transwell system to measure cytokine levels, glioma cell viability, and apoptosis rate in U87 glioma cells cocultured with induced M2 macrophages.

3. Results

Utilizing TIMER2.0 (http://timer.cistrome.org/) to analyze the expression difference of the IGF2BP1 gene between tumor and adjacent normal tissues in all tumors from TCGA. In GBM, the expression of the IGF2BP1 gene in tumor tissues was significantly higher than that in adjacent normal tissues ($P = 0.0087$) (**Figure 2A**).

Further analysis was conducted on the mesenchymal GBM in the TCGA database using the relevant genes for GBM subtype classification from the study by Chanoch-Myers *et al.* [32]. The results showed that patients without MGMT promoter methylation were older ($P = 0.024$) (**Figure 2B**); there was a positive correlation between IGF2BP1 expression and MYC gene expression ($P = 0.005$) (**Figure 2C**); patients with low IGF2BP1 expression had significantly better OS compared to those with high IGF2BP1 expression ($P = 0.036$) (**Figure 2B** and **Figure 2D**); using the CIBERSORT algorithm for immune infiltration analysis of these samples, the expression of "B cells native" and "Macrophage M0" are positively correlated with the expression of the IGF2BP1 gene ($P = 0.0073$, $P = 0.0395$) (**Figure 2E**), while the expression of "B cells memory" and "Macrophage M2" are negatively correlated with the expression of the IGF2BP1 gene ($P = 0.0108$, $P = 0.0048$) (**Figure 2E**).

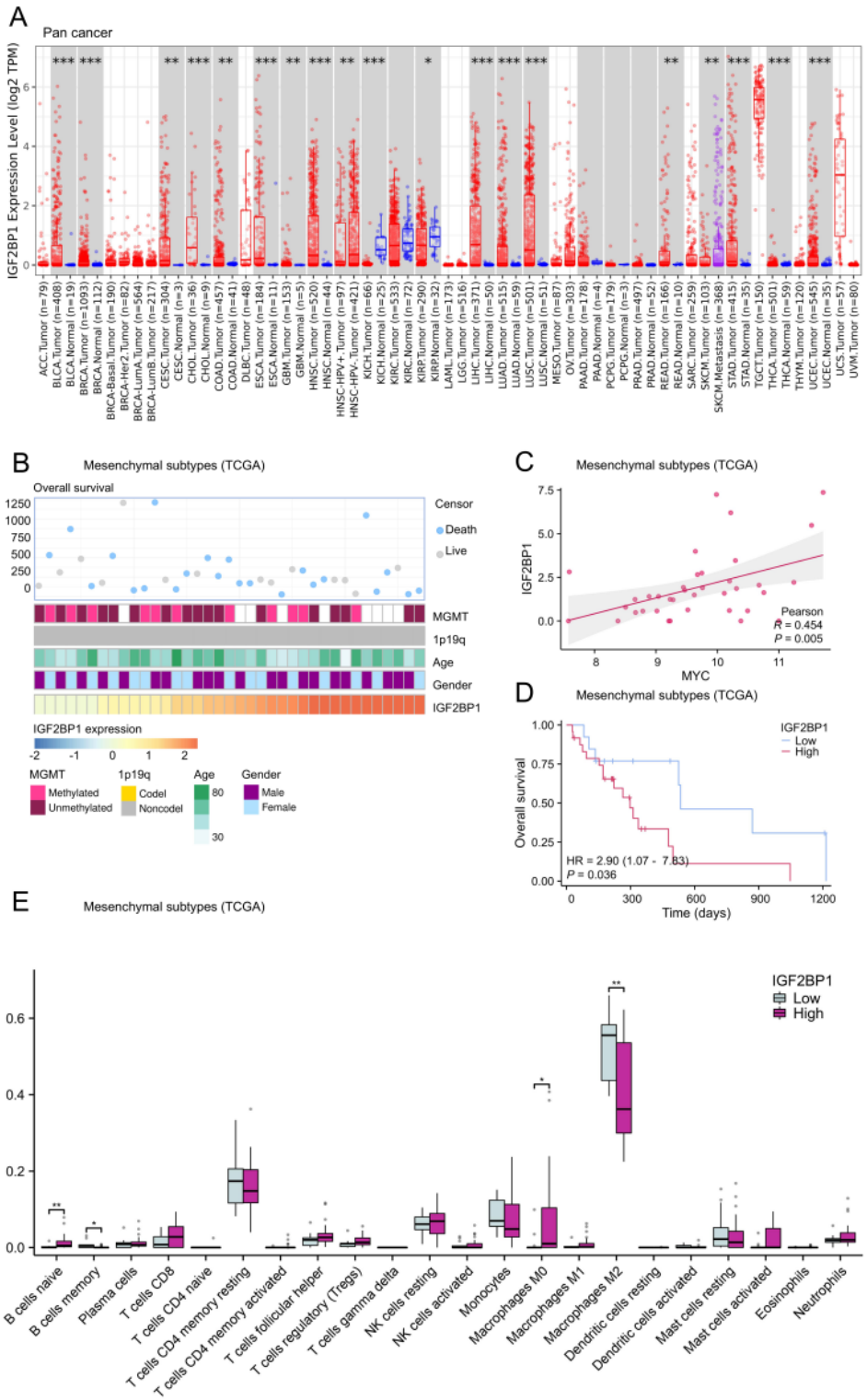

Figure 2. (A) Expression profile of IGF2BP1 across multiple cancer types; (B) Landscape of IGF2BP1-related clinicopathological features in the mesenchymal GBM from the Cancer Genome Atlas (TCGA) database; (C) Correlation between IGF2BP1 gene expression and MYC gene expression in the mesenchymal GBM from the TCGA database; (D) Prognostic analysis of IGF2BP1 gene expression in the mesenchymal GBM from the TCGA database; (E) Association between IGF2BP1 expression and immune cell infiltration in the mesenchymal GBM from the TCGA database.

Gene set variation analysis (GSVA) was performed on the immune cells related to this subtype to determine the enrichment scores for immune processes (**Figure 3**). Correlation analysis between the enrichment scores and IGF2BP1 expression revealed a positive correlation between IGF2BP1 expression and the immune functions of most B cells and macrophages.

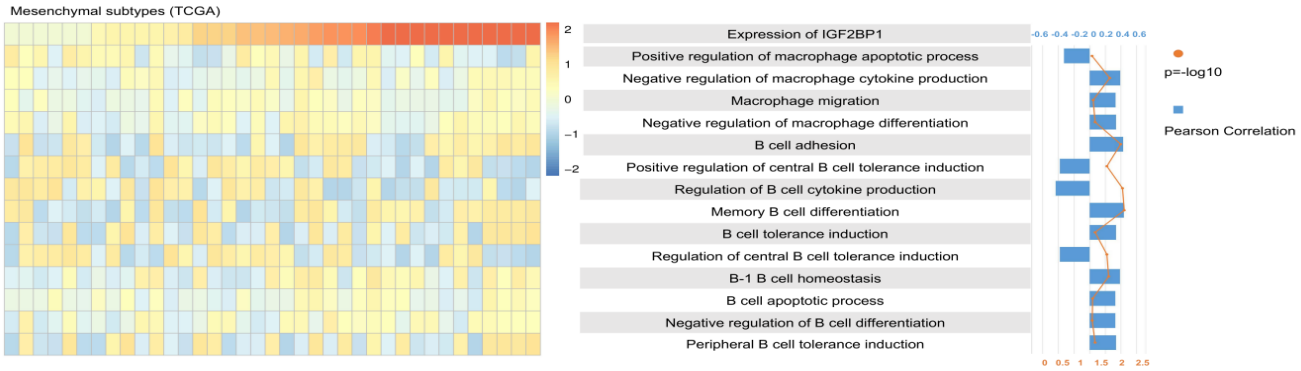

Figure 3. Gene set variation analysis of the mesenchymal GBM from the TCGA database.

4. Conclusion

Glioma, particularly GBM, is one of the most common malignancies of the Central Nervous System (CNS). Its highly aggressive nature and extremely high mortality rate have posed significant challenges in clinical treatment. The analysis of TCGA data revealed that IGF2BP1 expression is significantly elevated in glioblastoma (GBM) tumor tissues compared to adjacent normal tissues. Further investigation in the mesenchymal GBM subtype identified that patients without MGMT promoter methylation were older and that IGF2BP1 expression positively correlated with MYC gene expression. Notably, patients with low IGF2BP1 expression exhibited better overall survival (OS) than those with high expression. Immune infiltration analysis showed that IGF2BP1 expression positively correlated with “B cells native” and “Macrophage M0” but negatively with “B cells memory” and “Macrophage M2.” GSVA indicated a positive correlation between IGF2BP1 expression and the immune functions of most B cells and macrophages.

Current treatment methods are limited, and the immunoregulatory mechanisms within the Tumor Microenvironment (TME) are complex. The focus on the role of m6A RNA modification in GBM immune evasion may provide new insights for future immunotherapy approaches.

Disclosure statement

The authors declare no conflict of interest.

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