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Fibrinogen-like protein 2: its biological function across cell types and the potential to serve as an immunotherapy target for brain tumors

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Abstract

Brain tumors are among the 10 leading causes of cancer-related death and present unique treatment challenges due to their critical location and genetic heterogeneity and the blood-brain barrier. Recent advances in targeted immunotherapy and immune checkpoint blocking therapy provide alternative therapeutic strategies for brain tumors. Fibrinogen-like protein 2 (FGL2), which induces transformation from low-grade glioma to high-grade glioblastoma, is a type II membrane protein that is highly expressed in both host immune cells and tumor cells. Studies have uncovered multiple forms of FGL2 proteins with a broad range of roles in inducing immune tolerance and avoiding immune surveillance in tumor cells. Of note, presence of FGL2 transforms low grade to high grade brain tumors *via* promoting Treg, macrophages, and perhaps stemness. Absence (knockout) FGL2 in tumor cells (not in host cells) induces CD103 DC cells, which triggers tumor specific CD8+T cell activity to reject brain tumor progression. Immunotherapies targeting FGL2 have shown great promise in improving survival time in murine models. In this article, we will summarize the biological function of FGL2 in immune and tumor cells.

Keywords

Fibrinogen-like protein 2; brain tumors; immunotherapy

1. Introduction

Brain tumors refer to a group of neoplasms originating from the intracranial tissues and the meninges and account for about 90% of primary central nervous system tumors. The incidence of brain tumors varies by patients' age, gender, ethnicity, and tumors' histologic type. Primary brain tumors are highly heterogeneous and thought to develop through the

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accumulation of genetic alterations or mutations (Bondy, Scheurer et al. 2008, Ostrom, Cioffi et al. 2019).

The treatment of brain tumors depends on tumor types and locations and may include chemotherapy, radiotherapy, surgery, and, recently, immunotherapy. However, the clinical management of most brain tumors is made difficult by their significant heterogeneity and the existence of the blood-brain barrier (Park, de Lomana et al. 2021). To improve the prognosis of brain tumors, clinical trials and preclinical studies have focused on immunotherapies, including vaccines, therapeutic antibodies, and T cell therapy (Lyon, Mokarram et al. 2017). At this stage, seeking novel and specific immune therapy targets remains of great interest in brain tumor research.

The immune-suppressive protein fibrinogen-like protein 2 (FGL2) is known to be expressed in immune cells such as macrophages. However, recent studies found a much higher level of FGL2 expression in a subpopulation of glioblastoma (GMB) cells, and GBM is the most common aggressive malignant brain tumor in the United States (Yan, Zhao et al. 2019). This expression triggered alarms because FGL2 plays a crucial role in regulating the innate and adaptive immune responses. Indeed, knockout of the *FGL2* gene in tumor cells, but not in host cells. completely impaired tumor progression in brains (Yan, Zhao et al. 2019). This review will detail these recent discoveries of FGL2 with the purpose of exploring its potential as a target for immunotherapy in patients with brain tumors.

2. Transcriptional regulation sites for *FGL2* expression

The human *FGL2* gene is located at chromosome 7p and is 6450 bp in length. The gene structure is simple compared to most mammalian genes, with only one intron (2194 bp) located between two exons. This simple structure could suggest that gene expression regulation primarily occurs in the promoter of *FGL2*. The putative promoter region of the *FGL2* gene contains many cis-regulated element consensus sequences, including the standard TATA box and transcriptional binding sites, such as AP1 (Fos:Jun), C/EBP binding site (CAAT), Ets, and others (Levy, Liu et al. 2000) (Figure 1). Liu et al. demonstrated that the core promoter region was from −119 bp to −58 bp and the regulation region was from −1.3 Kb to 119 bp through serial 5′-deletions of *FGL2* promoter regions in RAW264.7 macrophage cells (Liu, Mendicino et al. 2006). However, the key regulating elements may depend on cell types because a different set of cis-regulated elements (Oct-1(POU2F1), Sp1/Sp3, ETS/GAS, and STAT-x sites) were utilized for constitutive expression of the *FGL2* gene in endothelial cells.

Regardless of macrophages and endothelial cells, IFN-γ appears to play an important role in regulating FGL2 expression (Liu, Leibowitz et al. 2003). However, this conclusion needs to be validated, at least in tumor cells, because FGL2 presence or absence in tumor cells—but not in the host cells—holds the key to tumor progression (Yan, Zhao et al. 2019).

3. Functional FGL2 protein formation

The FGL2 protein contains 439 amino acids in length, including a 23-amino acid signal peptide in the N-terminal and a 416-amino acid functional peptide. After translation, four

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65-kDa mature FGL2 monomers will form a 260-kDa homo-tetrameric structure linked by disulfide bonds, which play a critical biological function (Liu, Yang et al. 2013). FGL2 can function as either a membrane-anchored or an extracellularly secreted protein. Membrane-anchored FGL2s are tetramers, while secreted FGL2s could be tetramers, dimers, or monomers. Membrane-anchored FGL2 is a type II transmembrane protein with the N-terminal cytoplasmic part and the C-terminal extracellular domain. Three nearby amino acids (362, 364, and 366) of the FGL2 protein compose a Ca++ binding site in its C-terminal (Figure 2A). Close to the Ca++ binding site, five amino acids (371, 374, 375, 384, and 385) make up a polymerization pocket in each monomer, which may involve in the polymerizing formation of FGL2's C-terminal fibrinogen-related domain (FReD) (Protein[Internet] 1988, O'Leary, Wright et al. 2016). The membrane-anchored FGL2 could be cleaved to soluble FGL2 by an unknown mechanism. Based on the AlphaFold Protein Structure Database (Jumper, Evans et al. 2021, Varadi, Anyango et al. 2022), the FGL2 protein forms a coiled-coil domain in the N-terminal and a globular FReD in the C-terminal (Figure 2B). The coiled-coil structure consists of two alpha-helices that wind around each other to form a mechanical supercoil cable structure. The globular FReD is an essential immune functional domain demonstrating a comprehensive immunosuppression function through secreting soluble FGL2 in the central region of the C-terminal (Chan, Chan et al. 2002). The cysteines located in the N-terminal coiled-coil domain are involved in the formation of the tertiary or quaternary structure via inter-chain disulfate bonds, while four cysteines (206, 235, 364, and 377) located in the globular domain are related to maintaining the functional structure of FGL2 (Liu, Yang et al. 2013) (Figure 2C). In addition to cleavage, glycosylation impacts the biological function of FGL2 by affecting its stability. Five glycosylation sites affect protein turnover time, which could cover the cut sites of extracellular enzymes as well as increase water solubility and maintain structural stability (McNicholas, Potterton et al. 2011, Bienert, Waterhouse et al. 2017, Waterhouse, Bertoni et al. 2018) (Figure 2D).

4. FGL2 protein-producing tissues

FGL2 gene expression is affected by multiple factors, including cell types, stimulators, diseases, and others. According to The Human Protein Atlas (Uhlen, Karlsson et al. 2019, Sjostedt, Zhong et al. 2020, Atlas 2022), lymphoid tissues such as the spleen and lymph nodes have the highest expression; gastrointestinal tract organs such as the small intestine and colon and urinary system organs such as the kidney and urinary bladder have moderate expression levels. The presentation of FGL2 varies in other organs, such as lungs, breast, and muscles. *FGL2* gene expression differs across cell types. Monocytes, dendritic cells (DCs), and macrophages, including Kupffer and Langerhans cells, have maximum *FGL2* expression. The lymphoid cells such as T cells, B cells, and NK cells express low levels of *FGL2*, and skeletal myocytes, adipocytes, fibroblasts, and collecting duct cells have intermediate expression levels. Enterocytes, keratinocytes, alveolar cells, glia cells, and others. have a minimal level of *FGL2* expression (Supplementary figure 1). Rüegg et al. identified *FGL2* expression in the human small intestine via a specific probe to screen the cDNA library and obtained a clone encoding the *FGL2* gene (Ruegg and Pytela 1995). Two *FGL2* mRNA variants of around 1.5 and 4.5 kb have been found in resting peripheral blood T lymphocytes, including CD3+/CD4+ CD3+/CD8+ T lymphocytes.

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A study, in which the *LacZ* reporter gene β-galactosidase was knocked-in at the *Fgl2* gene loci to simultaneously express, indicated that β-galactosidase activity was mainly in lymphoid organs (Shalev, Liu et al. 2008). Examination of the gastrointestinal tracts confirmed that *Fgl2* was highly expressed in bone marrow, lymph nodes, spleen, and the lamina propria of the stomach and intestine.

5. The biological functions of FGL2 protein

FGL2 gene expression is associated with pleiotropic physiologic functions, especially prothrombinase activity and immune suppression. Membrane-bound FGL2 protein shows the activity of the prothrombinase complex, which converts fibrinogen into fibrin, resulting in local fibrin deposition that subsequently causes vascular thrombosis and tissue inflammation. The enzyme activity of FGL2 confirmed that FGL2 is a serine protease and is blocked by diisopropylfluorophosphate, a specific serine protease inhibitor. FGL2 needs accessories—phospholipids of cell membranes, free calcium, and coagulation factor Va—to activate the coagulation cascade. In mice, Fgl2 has three Ser-Xaa-Xaa-Lys (SXXK) motifs that can be catalyzed by serine peptidase clan E. In humans, there is only one SXXK motif near Ser91 (Chan, Chan et al. 2002). Based on a domain rich in glutamic acids that facilitates Ca2+ binding, Li et al. chose a 12-amino acid peptide in the human FGL2 sequence containing abundant glutamic acid residues near Ser91 to generate neutralizing anti-NPG-12 antibodies (Figure 3). The anti-NPG-12 antibodies showed the apparent capacity to inhibit the prothrombinase activity of FGL2 but did not affect the clotting time associated with the heparin. The anti-NPG-12 antibodies would not affect T lymphocyte proliferation and activation driven by anti-CD3/anti-CD28 monoclonal antibodies (Li, Wang et al. 2014). Levy et al. reported the correlation betweenFGL2 expression in macrophages and fibrin deposition in hepatic sinusoids. Microvascular thrombosis and hepatocellular necrosis resulted in progressive tissue necrosis in fulminant viral hepatitis (Levy, Liu et al. 2000).

Secreted FGL2 was found in culture supernatant in a tetramer format but lacked coagulation activity (Marazzi, Blum et al. 1998). Secreted FGL2 acts as a negative regulator of the immune response via its FReD. This domain shares high homology with the functional structure of fibrinogen-related immunoregulators such as tenascin (Ruegg, Chiquet-Ehrismann et al. 1989). Liu et al. created a serial FGL2-derived peptide library covering most parts of the FGL2 protein, primarily the FReD (Figure 3). Using a competitive mixed lymphocyte reaction assay, all peptides that showed significant blocking effects on FGL2 activity were located inside the FReD. The main interface of FGL2's interacting with its receptors were the five β-sheet planes and calcium/acetyl group-binding regions (Liu, Yang et al. 2013). Recombinant FGL2 was found to form a tetramer, which could bind to the low-affinity Fcγ receptors, FcγRIIB (CD32b) and FcγRIII (CD16), on antigen-presenting cells but not fibrinogen binding receptors such Mac-1 (CD11b/CD18) or TLR4. Through binding to FcγRIIB, recombinant FGL2 inhibited DC maturation in vitro and prolong skin allograft survival in vivo (Liu, Shalev et al. 2008). Absence of FGL2 does not impact hematological profiles such as bleeding times, prothrombin, and partial thromboplastin time (Marsden, Ning et al. 2003); however, the Fgl2-null mice presented

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lower fibrin deposition and liver necrosis and a higher survival rate after being infected with MHV-3.

5.1. FGL2 expression in T cells and its associated function

Soluble FGL2 has been shown to be constitutively secreted by CD3+, CD4+, and CD8+ T lymphocytes in peripheral blood, but no membrane-anchored FGL2 was detected (Marazzi, Blum et al. 1998). The secreted FGL2 comprised homologous poly-units in which the monomer was linked via disulfide bonds. Compared to the weak expression of *FGL2* in CD3+/CD45RA+ naive T lymphocytes, CD3+/CD45R0+ memory T lymphocytes preferentially expressed the *FGL2* gene. However, active stimulations and culture conditions would rapidly decrease the FGL2 expression and secretion in T lymphocytes without the presence of IFN-γ. FGL2 blockage caused the number of tumor-infiltrating CD8+ T cells to increase and improve their cytotoxicity against subcutaneously transplanted hepatocellular carcinoma. Compared with wild-type mice, more tumor-infiltrating CD8 T lymphocytes were activated to decrease the growth of orthotopically transplanted hepatoma in Fgl2-knockout mice (Yang, Zhang et al. 2019). Soluble FGL2 binds to T cells, resulting in the impairment of T cell proliferation under various stimuli (Chan, Kay et al. 2003). Regulatory T cells are a suppressive subset to protect the host from autoimmune disease, constitutively expressing FGL2 via a master transcription factor, FOXP3, although the *FGL2* gene is not a direct FOXP3-targeted gene (Gavin, Rasmussen et al. 2007, Zheng, Josefowicz et al. 2007). As a putative effector gene, *FGL2* is more highly expressed in CD4+CD25+ regulatory T cells than in CD4+CD25− T cells. The use of *FGL2* gene knockout or an FGL2-neutralizing antibody reverses the suppressive function of CD4+CD25+ regulatory T cells, resulting in the activation and proliferation of CD4+ T cells in cultures (Shalev, Liu et al. 2008). FGL2 acts as a function effector of FOXP3+ regulatory T cells and suppresses effector T cells' activities.

5.2. FGL2 expression in B cells and its associated function

FGL2 receptor FcγRIIB, but not FGL2, is highly expressed in human peripheral B cells with a hierarchical order of plasma cells (CD19+CD27+CD38+) > memory B cells (CD19+CD27+CD38−) > naïve B cells (CD19+CD27−CD38−), which indicates that B cells are potentially susceptible of FGL2 regulation. The activated FcγRIIB can reduce viability and block the in vitro differentiation of plasma cells, including the antigen-independent differentiation of B cells to plasma cells. The induced apoptosis and impaired proliferation of human B cells by FcγRIIB activation occur through the BTK and p38 MAPK pathway (Tzeng, Li et al. 2015). In a rat cardiac allotransplantation model, Fgl2 acted as a tolerogenic molecule to induce and enrich regulatory B cells inside the graft to improve long-term allograft survival (Bezie, Picarda et al. 2015). The tolerance was transferable and dominant allogeneic graft acceptance through the splenic tolerogenic cells. Furthermore, in Fgl−/− mice, dramatic increases were seen in type-1 and type-2 T cell-independent and T cell-dependent B cell responses to T cell-independent Ags, LPS, and NP-Ficoll compared to wild-type mice (Shalev, Liu et al. 2008). Together, these studies show that FGL2 has a pronounced regulatory effect on B cell function.

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5.3. FGL2 expression in monocytes/macrophages and its associated function

Not only is FGL2 expressed in macrophages, but the secreted FGL2 protein produced from tumor cells yields a strong impact on macrophage behaviors. In an Fgl2 knockout mouse tumor model, in which secreted Fgl2 protein was detected at a level comparable to that of human GBM cells, the percentage of CD11b+F4/80+CD49d+ P2RY12− macrophages was significantly higher (around 30% of brain-infiltrating leukocytes) than that (about 7%) in Fgl2-null tumors (Yan, Zhao et al. 2021). Tumor cells derived from FGL2-high tumor cells directly attracted macrophages, and an FGL2-neutralizing antibody dramatically decreased the chemoattractant effect of FGL2. By using the blocking antibodies to FGL2 receptors, FcγRIIb (CD32) and FcγRIII (CD16), the FGL2-driven migration of macrophages was significantly impaired, indicating that, in addition to suppression, FGL2 also acts as a potent chemokine to attract macrophages via its CD16a receptor. Activation of the FGL2-CD16-Syk-HIF1α signaling pathway in macrophages induces the secretion of CXCL7, causing the self-renewal and tumorigenicity of glioma cells.

5.4. FGL2 expression in DCs and its associated function

CD103+ DCs are crucial for inducing an anti-tumor T cell response (Roselli, Araya et al. 2019). In mice, Batf3-dependent cross-presenting CD103+ DCs were notably increased in Fgl2KO tumor cells compared to control tumor cells (Yan, Zhao et al. 2019). The Batf3-deficient mice showed a lack of both CD103+ DCs and CD8a+ DCs, indicating that Batf3 is a key factor in the activity of CD103+/CD8a+ DCs to trigger immune defense in Fgl2 function. Lack of Fgl2 rendered DCs more efficient and increased their capacity to present antigens and prime T cell responses. Fgl2 showed a strong capacity to impair DC development and differentiation from bone marrow cells in vitro via GM-CSF function.

Blockage of FGL2 was shown to promote TRAF6-NF-κB signaling, JAK2/STAT1/5 signaling, and p38 activation in the differentiation of bone marrow cells, which is important for CD103 expression in DCs (Chan, Kay et al. 2003). This observation conflicted with the earlier description in which FGL2 was thought to promote DC, but this conflict could be reconciled by considering the different DC types. As emphasized in this section, only CD103+ DCs were impacted by the absence of FGL2. Another explanation for the discrepancy is that these two studies were conducted in different model systems (in vivo vs in vitro) (Roselli, Araya et al. 2019).

5.5. FGL2 expression in brain tumor cells and its biological function in mouse GBM models

According to the analysis of TCGA data, *FGL2* is highly expressed in glioma. Approximately 72% of patients with low-grade glioma have two copies, and about 84% of patients with GBM have amplification or copy gain of the *FGL2* gene. The *FGL2*-high patients had a lower 5-year overall survival rate than the *FGL2*-low patients, indicating that *FGL2* expression is associated with disease grade and poor prognosis (Figure 4). Genetically engineered DBT-FGL2 cells, which forced high expression of the *FGL2* gene, vastly increased cell proliferation in vitro and tumor growth rate in vivo compared to GFP control cells (Yan, Kong et al. 2015). In immune-competent mice, *Fgl2* knockout in GL261 and LLC cells impaired tumor growth and prolonged survival time, although

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these tumor inhibition effects were dependent on CD8+ T lymphocytes (Yan, Zhao et al. 2019). In Ntv-a mice, PDGFB ligand-derived low-grade tumors, which recapitulate human low-grade glioma, Fgl2 overexpression boosted tumor growth and shortened survival times. Furthermore, Fgl2 was found to promote the transformation of low-grade tumors into high-grade tumors (Latha, Yan et al. 2019).

5.6. FGL2 expression in brain tumor cells vs. host immune cells: which is more significant in promoting tumor progression?

In GBM, the *FGL2* gene is confirmed to have heterogeneous expression. There is low or null expression of the *FGL2* gene in normal neuron cells, endothelial cells, and primary human peripheral blood monocytes. In GBM specimens, the *FGL2* gene was shown to be primarily expressed in the GFAP+ glioma subpopulation. CD45+ immune cells and CD31+ endothelial cells also expressed the *FGL2* gene, but only accounted for a small fraction in GBM tumors. Furthermore, expression of the *FGL2* gene in tumor cells promoted disease progression in vivo (Yan, Zhao et al. 2019).

Although the expression of FGL2 had no direct effect on stem-like properties of glioma cells in vitro, FGL2 boosted tumorigenesis and affect tumor-infiltrating leukocytes, which implied that FGL2 has the potential capacity to promote tumor growth and inhibit immune response in vivo. Macrophages were the most abundant cells in tumor tissue, accounting for up to 30% of the tumor tissue, and these macrophages promoted tumor cell proliferation, survival, and migration (Quail and Joyce 2017). Tumor-derived FGL2 was shown to directly promote the infiltration and activation of macrophages to secrete CXCL7 through the CD16/SyK/PI3K/HIF1α pathway (Yan, Zhao et al. 2021). Furthermore, CXCL7 increased the stem-like functionality, tumor incidence, and disease progression of glioma cells. Meanwhile, the FGL2-CXCL7 paracrine loop was related to patients' higher macrophage signature and poorer prognosis. Therefore, myeloid leukocytes were essential for FGL2-regulated tumorigenesis. Tumor cells interacting with tumor-infiltrating leukocytes via FGL2 could facilitate tumor progression and cause poor prognosis.

6. *FGL2 i*n human brain tumors and its impact on survival combined with other genetic mutations or genes' expression

Data from TCGA showed that the *FGL2* expression was associated with the expression of many genes in brain tumors. With the Spearman's rank correlation coefficient (ρ) set at 0.7 and adjusted *p*-values (*q*-value) at 10^{-10}, there were 196 genes in GBM, 127 genes in low-grade glioma, and 180 genes in diffuse glioma that strongly correlated with *FGL2* (Figure 5). Thirty-five overlapped genes showed in all three types of tumors; these genes mostly either promote tumor growth as an oncogene (e.g., *CTSS*, *EVI2B*, *SLC7A7*) or inhibit immune response as an immune checkpoint (e.g., *CYBB*, *C3AR1*, *DAPP1*) (Cerami, Gao et al. 2012, Hoadley, Yau et al. 2018, Barthel, Johnson et al. 2019) (Figure 6, Table 1). *FGL2* was highly expressed in the gliomas with chromosomes 1p and/or 19q deleted. It had an essential function in the malignant progression of high-grade glioma and was required for GL261 cells to transform to an aggressive status in vivo. Mesenchymal glioma stem cells, defined by *CD44*, *FN1*, *CHI3L1*, and *CTGF* expression, dramatically increased

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FGL2 expression compared to cells with the proneural subtype, defined by *OLIG*, *SOX2*, *SOX9*, and *PROM1* expression, indicating that FGL2 is associated with the mesenchymal subtype of high-grade glioma (Latha, Yan et al. 2019). According to the TCGA dataset, the expression of *FGL2* is also closely associated with *CSF2* and *CD8B* expression. High *CSF2* levels were concurrent with low FGL2 levels, indicating a more favorable prognosis for GBM patients (Yan, Zhao et al. 2019). In the low-grade glioma patients with a mutation of the isocitrate dehydrogenase gene (*IDH1*), the expression level of *FGL2* significantly affected survival time, but not in the patient without the mutation (Latha, Yan et al. 2019).

7. FGL2 antibody: immune therapy for brain tumors?

The recent studies confirmed that the FGL2 is an immunosuppressive hub that is significantly correlated with the expression of most immune inhibition molecules such as PD-L2, PD-1, CD39, BTLA, LAG3, IL-10, and TGFβ1. Treatment with a monoclonal antibody against FGL2 prevented necrosis, blocked viral replication, and promote the survival of MHV-3–infected mice in a dose-dependent manner compared to an isotype control antibody (Shalev, Wong et al. 2009).

Although a rabbit anti-Fgl2 polyclonal antibody did not statistically significantly reduce the level of FGL2 in the serum of GL261 tumor-bearing mice, it did effectively impair the functions of CD39+ regulatory T cells, M2 macrophages, and myeloid-derived suppressor cells, as well as reduce PD-1 expression through FcγRIIB, which dramatically prolonged the median survival of the mice (Yan, Kong et al. 2015).

Moreover, in an RCAS-PDGFB+FGL2 mouse model, the FGL2 polyclonal antibody reduced the number of CD4+FoxP3+ regulatory T cells and arginase 1+/Iba1+ macrophages, indicating it had the potential capacity to suppress M2 polarization and block regulatory T cell accumulation in the tumor environment. The anti-FGL2 polyclonal antibody-treated mice showed a significantly longer survival time than control mice (Latha, Yan et al. 2019).

Two anti-FGL2 monoclonal antibodies, F48 and F59, were developed to enhance the immune response and treat tumors. In a subcutaneous mouse model with GL261 GBM cells, the F48 antibody showed its capacity to suppress the M2 cell population in the tumors. Combined treatment with anti-FGL2 and anti-PD-L1 antibodies inhibited CT2A GBM growth in mice. Both F48 and F59 antibodies significantly improved mouse survival in GL261 glioma tumor model. The effect of the FGL2 antibodies was also confirmed on melanoma, Lewis lung carcinoma, and astrocytoma. In conclusion, the anti-FGL2 antibodies were effective at inhibiting tumor growth and preventing metastasis in several cancer models (LI 2018).

Though anti-FGL2 antibodies showed specific effects in some tumor models, their clinical use will be limited without competition screening to increase their affinity. However, as a crucial immune checkpoint in the tumor, FGL2 is a potential target for other tumor immunotherapies.

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8. FGL2-blocking T cell therapy

Recently, we developed engineered T cells, which were armed with self-created FGL2-blocking single-chain variable fragments (FGL2Nu-T). This membrane-anchored FGL2 neutralization antibody T cell was created by linking a signal peptide, scFv, peptide linkers, and an EGFR transmembrane domain. The combination of CAR-T and FGL2Nu-T cells rapidly reduced tumor volume in PDX sarcoma mice. And the combined treatments of FGL2Nu-T and cyclophosphamide also caused a decrease in tumor volume in an osteosarcoma mouse model. Furthermore, the FGL2Nu-T and doxorubicin combination almost completely shrank tumor volume. Following FGL2Nu-T therapy, tumor-specific memory T cells were observed in DBT brain tumors. Therefore, FGL2Nu-T treatment could act as a vaccine to induce tumor-specific memory T cells response and inhibit tumor growth (LI 2019).

9. Future FGL2-blocking strategies

The treatment of brain tumors remains a challenge for specific difficulties such as the lack of a particular neoantigen and immune privilege caused by the blood-brain barrier. Many strategies have been investigated to overcome these obstacles. Targeting microRNA downregulating immunosuppressive genes such as *CTLA4*, *PD-1*, and *FOXP3* dramatically boosted effector T cell function, induced tumor regression, and prolonged survival time in a mouse model (Wei, Nduom et al. 2016). Using microRNA to break immunosuppression has been assessed and successfully promoted therapeutic immune response in brain tumors (Heimberger, Gilbert et al. 2013). To safely open the blood-brain barrier, focused ultrasound was studied and shown to noninvasively and regionally disrupt function, demonstrating vast potential in treating brain tumors (Konofagou, Tung et al. 2012). More new techniques and knowledge in biology will be used in the immunotherapy of brain tumors soon. Therefore, because FGL2 is a critical immune checkpoint, FGL2-targeted microRNA and focused ultrasound-assisted delivery may also be developed as therapeutic strategies for brain tumors in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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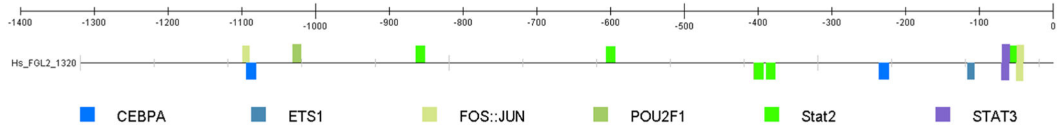

Figure 1.

The location of key transcription factor sites (analyzed and shown by the CiiiDER).

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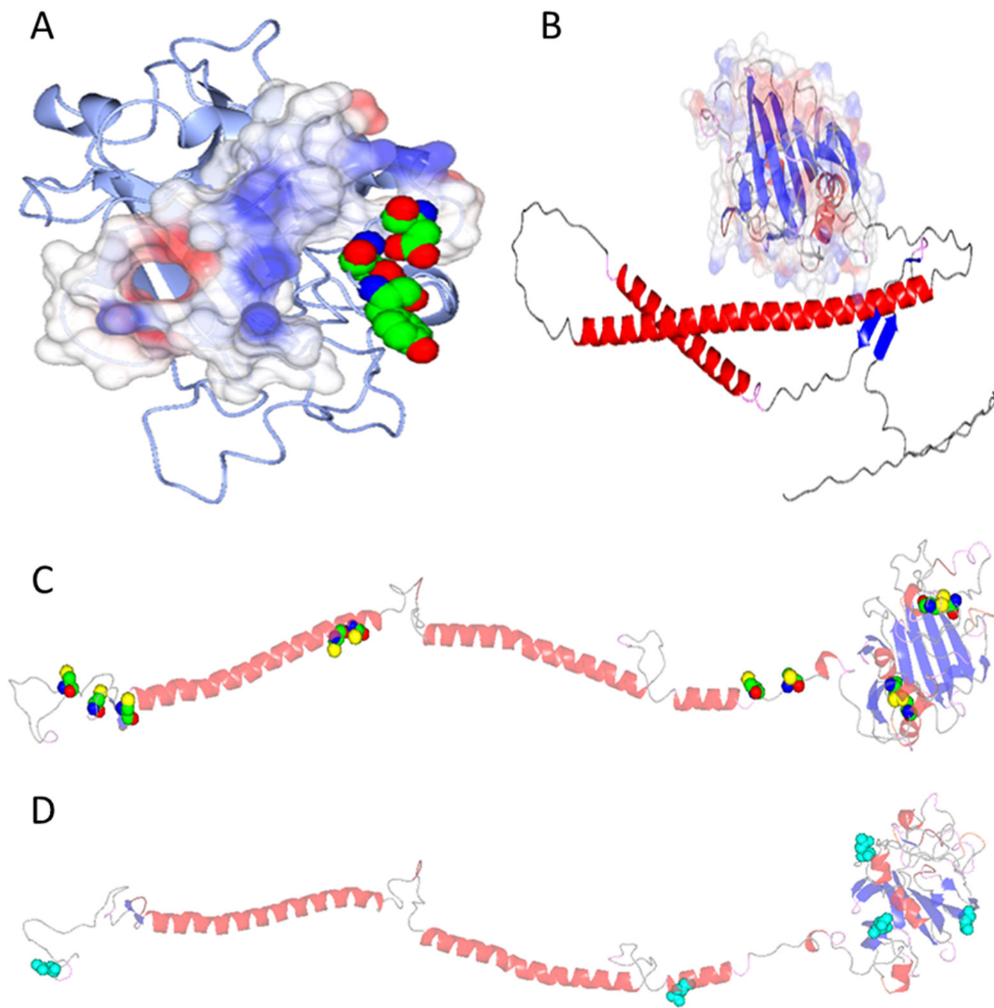

Figure 2.

The protein structure of FGL2. **(A)** The Ca^{++} binding interface (surface with electrostatic potential) of FGL2 and key three amino acids (sphere). **(B)** Retrieved molecule coordinates from the AlphaFold Protein Structure Database (alphafold.ebi.ac.uk) and displayed by the CCP4MG software. **(C)** The secondary structure, α-helix (red) and β-sheet (blue), and CYS (sphere) sites. **(D)** FGL2 glycosylation sites. The human FGL2 protein (NCBI RefSeq: NP_006673) was built with homology modeling (based on human fibrinogen γ - PDB: 3GHG) of the SWISS-MODEL and shown by the CCP4MG software.

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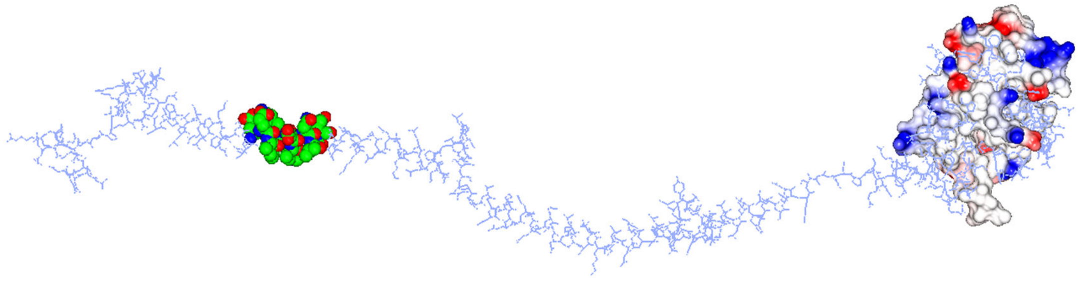

Figure 3.

The prothrombinase (left) and immunosuppression (right) functional domains in human FGL2. The human FGL2 (NCBI RefSeq: NP_006673) was built with the homology modeling (based on human fibrinogen γ - PDB: 3GHG) of the SWISS-MODEL and shown by CCP4MG software.

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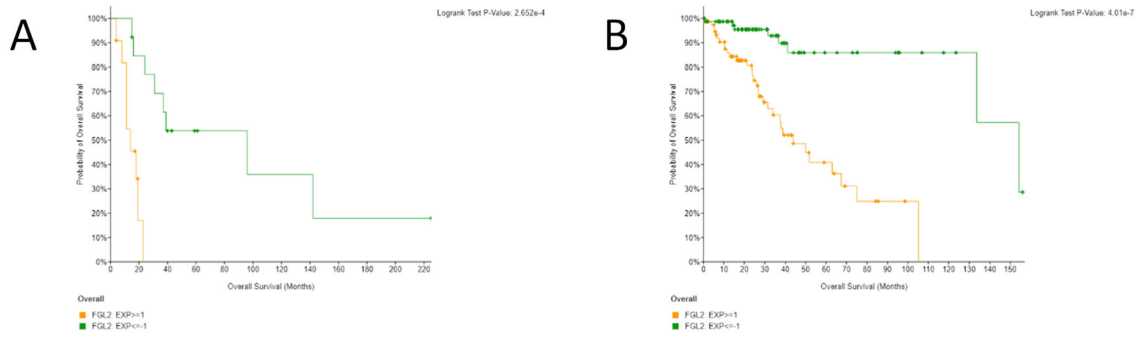

Figure 4.

The Survival curves of FGL2^{hi} and FGL2^{lo} patients with diffuse glioma **(A)** and low-grade glioma **(B)**. The results shown here are in whole or part based on data derived from or generated by the cBioPortal for Cancer Genomics https://www.cbioportal.org and TCGA Research Network: https://www.cancer.gov/tcga.

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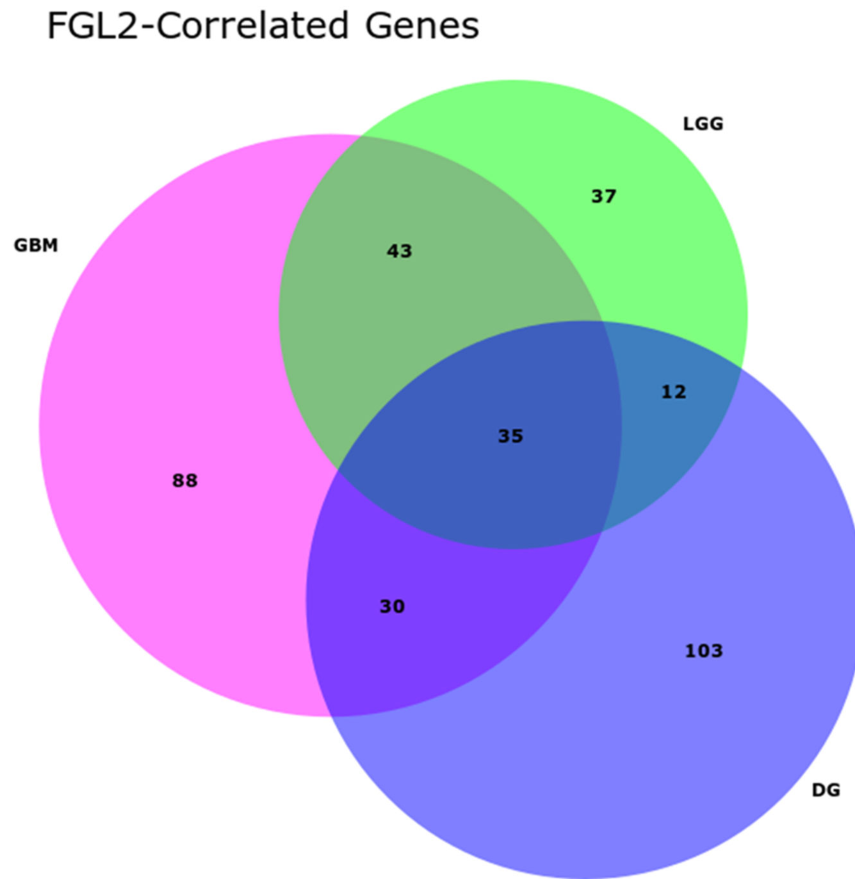

Figure 5.

The FGL2 strongly associated genes overlapped in GBM, low-grade glioma, and diffuse glioma (data derived from the cBioPortal for Cancer Genomics and drawn by Vennpainter)

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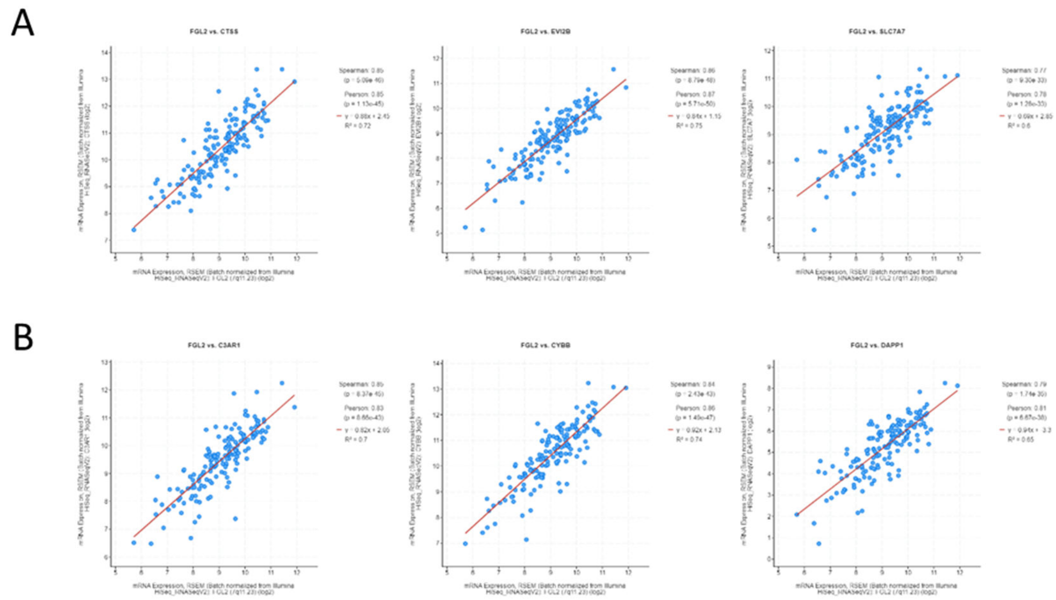

Figure 6.

The FGL2-associated oncogenes **(A)** and the FGL2-associated immune checkpoint genes **(B)**. The results shown here are in whole or part based on data derived from or generated by the cBioPortal for Cancer Genomics https://www.cbioportal.org and TCGA Research Network: https://www.cancer.gov/tcga.

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Table 1.

The gene list of 35 overlapped and strongly associated genes in brain tumors

PTPRC	CYBB	CD53	EMB	PLEK
CD86	TLR2	GPR65	FCGR3A	PIK3CG
EVI2B	TLR8	TLR1	SLC7A7	VNN2
CTSS	BTK	RNASE6	LAPTM5	CD69
C3AR1	GAPT	DAPP1	RCSD1	LCP1
RGS18	CLEC7A	LYN	CSF2RB	LHFPL2
TFEC	LCP2	MS4A6A	CCR5	KYNU