Targeting FGL2 in glioma immunosuppression and malignant progression

Xiaoyu Ma1, Hongtao Zhu1, Lidong Cheng1, Xin Chen2, Kai Shu1 and Suojun Zhang1*

1Department of Neurosurgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, 2Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Glioblastoma (GBM) is the most malignant type of glioma with the worst prognosis. Traditional therapies (surgery combined with radiotherapy and chemotherapy) have limited therapeutic effects. As a novel therapy emerging in recent years, immunotherapy is increasingly used in glioblastoma (GBM), so we expect to discover more effective immune targets. FGL2, a member of the thrombospondin family, plays an essential role in regulating the activity of immune cells and tumor cells in GBM. Elucidating the role of FGL2 in GBM can help improve immunotherapy efficacy and design treatment protocols. This review discusses the immunosuppressive role of FGL2 in the GBM tumor microenvironment and its ability to promote malignant tumor progression while considering FGL2-targeted therapeutic strategies. Also, we summarize the molecular mechanisms of FGL2 expression on various immune cell types and discuss the possibility of FGL2 and its related mechanisms as new GBM immunotherapy.

KEYWORDS
FGL2, resident immune cell, glioma microenvironment, immunotherapy, immune checkpoint molecules

Introduction

Gliomas are the most universal malignant central nervous system (CNS) tumors, accounting for approximately 80% of all brain malignancies (1, 2). According to the classification of central nervous system tumors in the 2021 World Health Organization (WHO), Adult-type diffuse gliomas are classified into three types: Astrocytoma, IDH-mutant; Oligodendroglioma, IDH-mutant, and 1p/19q-co deleted; Glioblastoma, IDH-wildtype (3, 4). Among these, glioblastoma (GBM) is one of the most lethal gliomas, accounting for 70% of all widespread glioma diagnoses, with a median survival time of 15 months (5). Currently, conventional treatments for gliomas include surgery, chemotherapy, and radiotherapy. However, the 5-year survival rate of GBM patients is
rarely 6.8% due to the infiltrative growth, aggressiveness, and recurrence of the malignant gliomas (6–8). The extremely low survival rate of GBM patients has prompted a search for more effective drugs and treatments (9).

Immunotherapy is an innovative treatment method in tumors today (10). The innate and adaptive immune system in the host recognizes and kills tumor cells in the initial stage of tumorigenesis, some tumor cells survive into the equilibrium the host recognizes and kills tumor cells in the initial stage of tumors today (10). The innate and adaptive immune system in the host's immune response and immune cells escape. It is based on the study of tumor immune cell escape mechanisms that immunotherapy has developed. Immunotherapy attempts to activate immune cells by reactivating the tumor immune system and blocking immune escape pathways (11, 12). Targeted cytokines, immune checkpoint inhibitors (ICIs), pericyte therapy, oncolytic viruses, and cancer vaccines are currently used in clinical treatment and have been verified to be helpful in fighting tumor cells (13). Due to the success of immunotherapy in melanoma and hematologic tumors, attempts have been made to understand GBM in terms of the immune microenvironment and immune response and to improve its prognosis (14). Unfortunately, CTLA-4 monoclonal antibody Ipilimumab, PD-1 monoclonal antibody Nivolumab and so on have shown in clinical trials limited efficacy against GBM (15).

Due to the complex immune microenvironment of glioma, immunotherapy of glioma has been increasingly studied in recent years, and some promising results have been achieved. (16). In brain tumors, tumor cells secrete a large number of cytokines, growth factors and chemokines that promote the entry of numerous non-tumor cells such as infiltrating immune cells, pericytes, astrocytes, oligodendrocytes, and endothelial cells into the tumor (16–18). These cells and the various factors constitute the tumor microenvironment, which plays a crucial role in promoting tumor development, metastasis, and resistance to cancer therapy. The complex immune microenvironment of glioma makes it an excellent challenge for immunotherapy (19). In order to evade immune surveillance and clearance, tumor cells can suppress anti-tumor immunity through cancer immune editing, by which the immune response shifts from preventing the development of cancer to promoting the growth of tumor cells, thus evading immune surveillance (20, 21).

FGL2 is an important pleiotropic immunomodulatory cytokine discovered in recent years, which in cancer achieves immunosuppression by inhibiting antigen-presenting cells (APCs), suppressing T cell proliferation, inducing macrophage polarization to M2 and inducing regulatory T cell (TREG) activity (22, 23). However, the role of FGL2 in TME and the therapeutic potential of targeting this cytokine in gliomas is unclear. Here, we review the role of FGL2 in brain cancer and discuss its role in immunosuppression and tumor progression. We examined the molecular mechanisms responsible for regulating FGL2 expression in various cell types and considered the possibility that immunotherapies developed against this cytokine may improve the prognosis of GBM patients.

Biogenesis of FGL2

Fibrinogen-like protein 2 (Fgl2), as a number of the fibrinogen-associated protein superfamily, has a molecular weight of approximately 64 KD (24). It was first identified in the 1990s and was initially thought to be secreted by a constitutively expressed cytotoxic T lymphocyte. The protein encoded by FGL2 is homologous to the β and γ chains of fibrinogen at its carboxy terminus and carboxy terminus, with a FRED structure (25). Numerous studies surrounding FGL2 have revealed many functions of it. For example, Th1 and Th2 cytokines trigger the coagulation system by acting on FGL2 on endothelial cells, FGL2 can directly activate prothrombin to produce thrombin. However, FGL2, which is secreted by peripheral blood CD4+ and CD8+ T cells, has no coagulation activity (26). It can inhibit the maturation of dendritic cells and the proliferation of T cells and has immunosuppressive activity. Due to the two activities exhibited by FGL2, it is now more uniform to categorize FGL2 as a membrane-bound protein and a secretory protein, with the membrane-bound type have coagulation activity, and the secretory type with immunomodulatory function (27, 28). Previous studies have shown that FGL2 plays an important role in inflammatory diseases such as severe viral hepatitis, rheumatoid arthritis, chronic obstructive pulmonary disease (COPD) (28), and inflammatory bowel disease (IBD) (29). At the same time, FGL2 is implicated in the malignant progression of tumors in hepatocellular carcinoma, central nervous system tumors (22), breast cancer, ovarian cancer and so on.

Recent studies have shown that FGL2 can contribute to the growth of gliomas by inducing multiple immune mechanisms (30). However, the role of FGL2 in gliomas and the therapeutic potential to target this protein in cancer patients remains unclear. Therefore, this paper reviews the role of FGL2 in glioma and discusses its role in immunosuppression and malignant progression. We summarize the molecular mechanisms by which FGL2 may be expressed in various cell types and explore the potential prognostic value and immunotherapeutic targeting of FGL2 in gliomas.

FGL2 and glioma

Yan et al. were the first to identify the strong expression of FGL2 in gliomas and conducted experiments to prove it (30). They found that 83.8% of GBM patients had FGL2 gene amplification or copy increase, and 72.5% of LGG patients had...
diploidy of the FGL2 gene. They divided GBM patients into FGL2 High and FGL2 Low groups according to FGL2 mRNA expression levels. The results showed that the overall survival rate of patients in the FGL2 High group was lower than that of patients in the FGL2 Low group. The median survival times for low and high expression of FGL2 were 394 and 357 days. The 5-year survival rate was estimated to be 4.98% for patients with low FGL2 expression and 0.99% for patients with high FGL2 expression. Song et al. found by immunohistochemistry that the expression of FGL2 was significantly higher in glioma tissues than in normal tissues (31). And we also found that FGL2 mRNA expression levels were higher in high-grade gliomas than in low-grade gliomas, and low expression of FGL2 increased patient survival and prolonged patient survival time based on the Chinese Glioma Genome Atlas (CGGA) (http://www.cgga.org.cn/) and the Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov/) databases. (Figure 1) These data suggest that FGL2 expression in glioma is positively correlated with tumor malignancy and patient survival.

Although the cellular origin of FGL2 in gliomas has not been determined, it has been shown that FGL2 is higher expressed in glioblastoma and glioblastoma stem cells (GSCs) compared to fibroblast cells. In contrast, there is no significant difference in FGL2 expression between glioblastoma and GSC line (22, 31). Using murine glioma models, Yan et al. verified that FGL2 might promote glioma growth in murine models by inhibiting the infiltration of immunosuppressive cells in the tumor microenvironment and that neutralization of FGL2 protein using anti-FGL2 antibody prolonged the survival time of mice (30). FGL2 secreted by GSCs is thought to activate tumor-infiltrating immune cells. For example, Fgl2 can immunomodulate the organism by inhibiting T cell differentiation and proliferation, DCs maturation, CD8+ T cell function, complement activation and promoting B cell apoptosis, etc. (22, 23, 30, 31). In addition, FGL2 overexpression can skew macrophages and other activated antigen-presenting cells (APCs) in the tumor microenvironment from an inflammatory (M1) or neoplastic (M0) phenotype to an immunosuppressive (M2) phenotype (23). The limitation of the current study is that the exact mechanism of FGL2-induced immunity is unclear and needs further investigation.

**FGL2 and tumor progression**

As well as its immunomodulatory functions of immunosuppressive and immunostimulatory activities, FGL2...
can promote tumor progression by increasing cancer cell proliferation (32). In the mouse model of lung cancer, FGL2 promotes tumor growth by stimulating angiogenesis, immunosuppression, and tumor cell proliferation (33). Similarly, in vitro studies showed a dose-dependent increase in FGL2-mediated cell proliferation in LOVO and SW620 colorectal cancer cells, and knockdown of FGL2 resulted in reduced proliferation, migration and invasion (34, 35). Overexpression of FGL2 can eliminate the decrease in cell proliferation, migration, and aggression caused by the MAPK signaling inhibitor U0126 (34). In hepatocellular carcinoma studies, investigators used recombinant hFGL2 protein to stimulate HCCLM6 cells with thrombospondin and Ca2+, then found that phosphorylated p38 and ERK were significantly upregulated, while this upregulation could be abrogated by hirudin (36). Stable downregulation of FGL2 expression in the FGL2 knockdown HCCLM6 cell line was found to result in delayed tumor growth and reduced angiogenesis, along with decreased VEGF I and II-8 expression. These findings suggest that FGL2 may regulate HCCLM6 tumor cell growth by affecting angiogenesis (36). However, this phenomenon was not reproduced in vivo.

Interaction of FGL2 with resident immune cells in glioma

Although the origin of FGL2 in glioma is not fully understood, it has been shown to be expressed mainly in immune cells such as endothelial cells, macrophages, NK cells, T cells and tumor cells (27, 37). Low levels of FGL2 expression are associated with high granulocyte-macrophage (GM-CSF) colony-stimulating factor expression (38). Overexpression of FGL2 in a mouse glioma model was observed to increase CD4+FoxP3+Tregs and induced macrophages toward M2 phenotype shift. Ultimately, T cell initiation capacity was decreased (30). Yan et al. found that knockdown of FGL2 in immunoreactive mice reversed immune dysfunction in dendritic cells (DCs) and induced differentiation of CD103+ DCs in the brain (37). In addition, FGL2 has also been shown to play an important role in regulating myeloid-derived suppressor cells (MDSCs) (39, 40). FGL2 was also found to have a strong correlation with both immune cells in the TIMER database. (Figure 2) These findings suggest that FGL2 exerts its immunosuppressive effects through a variety of tumor-mediated immunosuppressive mechanisms (Figure 3).

FGL2 inhibits DC maturation

Among the immune cells involved in the immune response, dendritic cells (DC) are the most functional antigen-presenting cells known (41–44). It can effectively extract, process, treat, and present antigens to T and B lymphocytes to activate the body’s specific immunity (45). DC presents a large number of tumor antigen peptides so that the corresponding T cell receptors are fully occupied; binds to T cells, facilitating T cell clearance of tumors; promotes T cell enrichment and enhances activation of T cells; fully activates T cells (46). Immature DCs can be released into tissues via blood vessels and are constantly searching for invading antigens and mutated cells. Once found, DCs immediately function to engulf these antigens and cells while digesting them with digestive enzymes from intracellular lysosomes. Digested foreign bodies and cells leave behind fragments of biomolecules that DCs will put on MHC molecules on the cell surface and enter the lymphoid tissue for T cell recognition (47–49). In this process, DC cells are gradually transformed into mature antigen-presenting cells, a process often referred to as “antigen presentation”. And studies in recent years have shown that DC cells are closely linked to the prognosis of tumors (50). In solid tumors, patients with a high number of DC cell infiltrations tend to have a better prognosis (51). Therefore, DC cells are also used in research for tumor treatment, such as DC vaccines (52).

The most abundant and intrinsically stimulatory migrating cell type is the CD103+ DC population. In melanoma, CD103+ DCs transport tumor antigens to Draining lymph nodes (dLN) in a CCR7-dependent manner, resulting in increased numbers of tumor-specific CD8+ T cells (53). FGL2 promotes glioma progression by inhibiting CD103+ dendritic cell differentiation (22). In tumors, tumor cells release antigen to bind with antigen-presenting cells APCs, which further activate T cells, and activated CD8+ T lymphocytes exert the ability to recognize and kill tumor cells (54). APCs express major histocompatibility class II (MHCII) molecules, including DCs, macrophages, monocytes, B lymphocytes, and microglia in the brain (55). Batf3 is a key transcription factor that regulates CD103+/CD8α+ DC differentiation (56). The study by Quintana et al. shows that in the absence of Batf3, there is an impairment in the recognition of tumor cells by T cells. GM-CSF promotes the differentiation of myeloid cells and plays an important role in the differentiation, proliferation and activation of DCs (57). Yan Jun et al. found that GM-CSF induced TAK1/NF-kB, p38 and JAK2/STAT3/STAT1 signaling to induce CD103 expression. And a small amount of FGL2 expression in tumor tissues could significantly reduce the intensity of these CD103+DC-induced signals (22). This discovery may shed new light on the development of DC immunotherapy.

FGL2 and tumor-associated T cell

T cells can shape immune responses in tumors, autoimmunity and infections, where CD4+ T (Th) cells and CD8+ T cells mediate effector responses that are suppressed by regulatory T (Treg) cells (58, 59). The balance between the effector T cell and
Treg cell functions coordinates immune homeostasis and regulation of immune function. It has a vital role in tumorigenesis and development. Depending on the information the environment delivers, T cell metabolism dynamically changes and determines various aspects of its functional differentiation. CD4+ or CD8+ T cells recognize tumor antigens and autoantigens by expressing T cell receptors (TCRs) and therefore play a crucial role in shaping the immune response in the formation of cancer or autoimmune diseases (60, 61). Following stimulation by cognate antigens, T cells are activated, proliferate, and undergo functional differentiation in response to environmental information. Initial CD8+ T cells without antigen stimulation differentiate into cytotoxic effector cells and long-term memory cells (62, 63). Initial CD4+ T cells differentiate into Th1, Th2, Th17, and Th effector cells, which can also form long-term memory cells, and immunosuppressive Treg cells expressing Foxp3 (64, 65). Cancer development is often associated with an immunosuppressive tumor microenvironment (TME), in which tumor-specific cytotoxic CD8+ T cells are often inadequate or dysfunctional and unable to eradicate malignant cells. T cell activation requires APCs such as CD103+ DCs to present antigens to T cells, while FGL2 can inhibit APC maturation and subsequently reduce T cell activation, the use of anti-FGL2 antibodies to antagonize FGL2 can activate T cells. Therefore, the development of FGL2 inhibitors may play an important role in the immunotherapy of gliomas (37).
FGL2 is an effective molecule of Tregs

Regulatory cells (Tregs) are a subpopulation of T cells that controls autoimmune reactivity in vivo, first reported by Sakakuchi et al. in 1995 (66, 67). Tregs can be divided into nTregs and aTregs. nTregs are mainly composed of Foxp3+CD25+CD4+ Treg cells, which function through cell-to-cell contacts (68). In recent years, many scholars have conducted numerous studies on the immunological aspects of Tregs in tumors. These studies confirm the large amounts of Tregs infiltration in tumor tissues such as liver (69), ovarian (70), lung (71), breast (72), and gastrointestinal tumors (73), and their significant presence is often associated with poor tumor prognosis. Attempts have been made to explain the mechanisms by which Tregs suppress the immune response at various cellular and molecular levels (74). They include: 1, through cell-to-cell contact-dependent inhibition, Tregs cells are inhibited by CTLA-4 and TGF-β etc. directly binding to the corresponding receptors on target cells, reducing the responsiveness of target cells to IL-2 and thus inhibiting the proliferation of effector T cells. 2, relying on suppressive cytokines such as IL-4 and IL-10. 3, modulating the body’s immunity by interacting with antigen-presenting cells, suppressing effector T cells by downregulating the function of APC cells or competing for co-stimulatory molecules on APC cells (75–82).

FGL2, which has immunomodulatory activity, has been reported to be highly expressed in Treg by many studies (83–85). To verify the importance of FGL2 on Treg function, Itay Shalev et al. used an anti-FGL2 monoclonal antibody to antagonize Treg in vitro. The experimental results showed that the FGL2 antibody could completely inhibit Treg’s function. This result further supports the previous finding that FGL2 may inhibit T cell proliferation by suppressing the production of immune-activating cytokines IL-2 and IFN-γ and promoting the production of suppressive cytokines IL-4 and IL-1 (25, 26).

FGL2 promote M2

Tumor-associated macrophages (TAM) are infiltrating macrophages in tumor tissues, which are mainly differentiated from monocytes (86, 87). Chemokines such as CSF1 and CCL2 secreted by tumor cells can recruit monocytes from peripheral circulating blood to the tumor microenvironment (TME), and then monocytes differentiate into macrophage (88, 89). A growing number of studies have shown that TAM has a series of tumor-promoting functions such as supporting tumor cell proliferation, invasion, and metastasis, and is highly correlated with poor prognosis of tumor patients (23, 90, 91). Based on phenotype and function, macrophages can be divided into two main types, M1 (pro-inflammatory, classically activated macrophages) and M2 (anti-inflammatory, alternatively activated macrophages) (92–94). In addition, M1-type macrophages kill tumor cells and defend against pathogen invasion, while M2-type macrophages mainly play a role in promoting tumor growth, invasion and metastasis (95, 96).
In gliomas, TAMs include brain-resident microglia and bone marrow-derived macrophages, which account for 50% of GBM tumors (97). TAMs and glioma cells are important components in promoting glioma growth, and their interactions are critical for glioma proliferation, treatment resistance, and tumor recurrence (98). FGL2 may be a central effector molecule in this interaction. Yan et al. found differences in macrophage infiltration between FGL2hi and FGL2KO gliomas by flow cytometry analysis, demonstrating that FGL2 can increase the M2 macrophage population. Macrophage migration assays were performed by using FGL2hi and FGL2KO glioma cells conditioned medium (CM). It was found that CM from FGL2hi attracted more macrophage aggregation than that from FGL2KO. The migratory effect of macrophages was also significantly suppressed after blocking FGL2 or using antibody blockers of the FGL2 receptor FcγRIII (CD16) (23). These findings suggest that FGL2 acts as a potent chemokine that recruits macrophages into the tumor microenvironment (TME) of gliomas, and CD16 is the receptor that mediates this chemotactic effect. They also found that FGL2 secreted on glioma cells could bind to the receptor CD16 on TAMs and promote the Syk/Pi3K/AKT/HIF1α signaling pathway, then induce the release of CXCL7, which in turn could act on glioma cells and facilitate their Stem-like transition (23). However, the relationship between FGL2, TAMs and gliomas cells has not been sufficiently studied, and more experiments are needed to verify the relationship in the future.

**Interaction of FGL2 with immune checkpoint molecules**

The current treatment of the new GBM diagnosis is still focused on maximal resection and combined radiation and chemotherapy. Unfortunately, almost all treated patients relapse within a short period, and there are currently no effective treatments to prolong their survival for relapsed patients (99, 100). So, it is urgent to find a more efficient treatment. Over the past few years, immune checkpoint inhibition has gradually received more attention in treating tumors, providing a ray of hope for some tumors with limited traditional treatment options (101, 102). For example, PD-1 (Programmed cell death protein 1) checkpoint inhibitor can bind to PD-1 or its ligand PD-L1 and block PD-1 from blocking T cell (103). But the current effect of immune checkpoint inhibitors represented by PD-1 in the treatment of glioma is not very satisfactory (104, 105). Therefore, it is essential to find more effective immune targets. In a review of previous studies, we found that FGL2 acts more on antigen-presenting cells APCs upstream of T cells. These cells are present in the glioma microenvironment (37), and play a key role in immunosuppression by suppressing T cell proliferation. As mentioned repeatedly in the literature, targeting a single molecule or pathway is not sufficient to inhibit the malignant progression of gliomas, so if we can find a key point that can inhibit multiple immunosuppressive pathways or mechanisms, it is possible to achieve good therapeutic results. As a molecule with such a role, the development of inhibitors of FGL2 may help reverse immunosuppression in the tumor microenvironment and may play an essential role in targeting glioma-mediated immunosuppressive therapy. Combined with previous findings, PD-1 can elevate FGL2 expression by inhibiting IFN-γ and TNF-α. It is not difficult to infer that simultaneous inhibition of IL-10 and PD-1 can increase T cell proliferation, which will be verified in the future (Figure 4). Ntv-a mice injected with RCA-PDGFB+FGL2 were treated with anti-FGL2 antibodies, and the control mice were treated with IgG antibodies. Statistical analysis of the survival of the mice revealed that the median asymptomatic survival of the anti-FGL2 antibody-treated mice was significantly longer than the control. The tumors treated with anti-FGL2 antibody were also found to have lower CD44 expression and higher Olig2 expression than the control group, indirectly demonstrating that high expression of FGL2 is associated with the mesenchymal subtype of glioma. Comparing the number of Treg in the two groups after 20 days of treatment, it was found that the anti-FGL2 antibody-treated mice showed fewer Treg along with a small number of arginase1+/Iba1+ macrophages. These suggest that anti-FGL2 antibody treatment can effectively prevent immunosuppression of the tumor microenvironment (106).

**FGL2 is involved in the formation of blood vessels in gliomas**

Liu et al. established a stable knockdown of FGL2 in the HCCLM6 cell line and found that downregulation of FGL2 reduced tumor angiogenesis in HCCLM6 nude mouse xenograft. hFGL2 expression in HCC tumor cells promotes tumor growth and angiogenesis through activation of ERK and JNK pathways, and FGL2 protein secreted by tumor cells promotes angiogenesis and tumor growth through activation of the thrombin-dependent MAPK pathway. Extensive studies have shown that IL8 and VEGF are the most important activators of tumor-associated angiogenesis (107–109). Activation of multiple VEGF/VEGF receptor signaling pathways leads to endothelial cell survival, mitosis, migration, differentiation, vascular permeability, and endothelial progenitor cell mobilization (110, 111). IL-8 exerts its powerful angiogenic properties on endothelial cells through interaction with its receptors CXCR1 and CXCR2 (112, 113). In the nude mouse subcutaneous tumor model with stable downregulation of FGL2, FGL2 was found to be downregulated along with reduced expression of VEGF and IL-8 (28). At the same time, downregulation of FGL2 was found
to affect angiogenesis in corneal microcapsule analysis in nude mice. However, FGL2 is also expressed in endothelial cells and macrophages, and FGL2 may also promote tumor angiogenesis by inducing value-added endothelial cells and recruiting inflammatory cells, this part needs to be further investigated. The correlation of FGL2 expression with enhanced phosphorylation of ERK and JNK strongly suggests that FGL2 plays an important role in tumor growth in HCC by regulating the activation of the MAPK pathway (36). FGL2 can lead to phosphorylation of ERK and p38 through thrombin production and subsequent activation of PAR1 and PAR3, or JNK through activation of PAR2, and these late cellular activities promote survival and proliferation, tumor growth and angiogenesis in hepatocellular carcinoma. These observations suggest that FGL2 prothrombinase, in conjunction with thrombin and tissue factor, may contribute to tumor hypercoagulability and possibly to angiogenesis and metastasis. In turn, FGL2 may serve as a novel target for the intervention of tumor development. Nevertheless, there are no relevant literature reports on whether FGL2 has an effect on tumor angiogenesis in glioma, and more experimental validation may be needed at a later stage.

**Conclusion**

FGL2 is widely expressed in gliomas and also plays an essential immunosuppressive role, thereby promoting tumor immune escape and malignant progression. Since FGL2 can act on antigen-presenting cells and inhibit the anti-tumor activity of T cells, it may be a future direction to try to apply FGL2 targeting therapy to GBM treatment. In-depth understanding and elucidation of the transcriptional and signaling mechanisms of FGL2 in different cell types of gliomas are essential for developing new specific targeted drugs.

**Author contributions**

SZ designed and led the project. XM wrote the manuscript. HZ, LC, XC, and KS provided valuable revisions to the manuscript. All authors contributed to this article and approved the submitted version.

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**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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