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Cyclooxygenase-2 in glioblastoma multiforme

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Abstract

Glioblastoma multiforme (GBM) represents the most prevalent brain primary tumor, yet there is a lack of effective treatment. With current therapies, fewer than 5% of patients with GBM survive more than 5 years after diagnosis. Mounting evidence from epidemiological studies reveals that the regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) is correlated with reduced incidence of GBM, suggesting that cyclooxygenase-2 (COX-2) and its major product within the brain, prostaglandin E₂ (PGE₂), are involved in the development and progression of GBM. Here, we highlight our current understanding of COX-2 in GBM proliferation, apoptosis, invasion, angiogenesis, and immunosuppression by focusing on recent in vitro and in vivo experimental data. We also discuss the feasibility of COX-2 as a therapeutic target for GBM in light of the latest human studies.

Keywords

cyclooxygenase; glioblastoma; glioma; neuroinflammation; prostaglandin; tumorigenesis

Introduction

GBM, also known as astrocytoma grade IV, is a highly infiltrating and aggressive tumor of the central nervous system (CNS) arising from the irregular astrocytes or other glial cells. GBM is the most common and devastating primary malignant brain tumor, accounting for 45.2% of malignant and 15.6% of all primary brain tumors, with an incidence rate of approximately 1 in 30 000 [1]. The median survival of patients with GBM with treatment is only approximately 15 months, and the 2-year survival rate is fewer than 25%. Without any treatment, most patients with GBM only can survive for a few months [2].

Pathophysiologically featured by necrosis, vascular proliferation, and mitotic activity, GBM causes a diversity of debilitating symptoms, including nausea and vomiting, aphasia,

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hemispatial neglect, visual field defect, cognition changes, gait imbalance, urinary incontinence, blurred vision, headache, memory loss, hemiparesis, and personality changes [3]. In addition, up to 60% of patients with GBM develop simple partial, complex partial, or generalized seizures, depending on the tumor loci. GBM-associated seizures represent a major obstacle to the tumor management and approximately 30% of patients can develop pharmacoresistance [4,5].

Owing to the blood–brain barrier (BBB), which restricts the infiltration of most antitumor drugs into the CNS, the standard treatment of GBM is limited to surgical resection followed by radiotherapy in combination with temozolomide [6]. However, the overall outcome of surgical treatment for GBM is often compromised by the complexity of intracranial operation, the extent of resection, and residual tumor cells that can cause tumor recurrence with a relatively short relapse time. Moreover, there are many cases where the surgical ablation is not an option because of the tumor location, tumor size, and/or poor patient performance status [7]. All these contributory factors together render the GBM as the most lethal as well as the most difficult-to-treat brain tumors. Developing new therapeutics for this devastating neurological condition is an urgent unmet need.

Despite continuous efforts in genomic studies, including those from the Cancer Genome Atlas (TCGA) projects that pinpointed several gene mutations and core pathways in GBM tumors, providing valuable insights into the biology of this disease [8,9], the molecular mechanisms underlying the development and progression of GBM largely remain unknown. Recent studies on animal models and human patients have revealed some important molecular events that are highly correlated with, and very likely contribute to, the growth, migration, angiogenesis, and immune evasion of malignant gliomas. Among these, COX-2 has been widely reported to be overexpressed in brain tumors [10–12], and has been proposed to facilitate the formation and invasion of glioblastomas. Here, we highlight our current understanding of COX-2 in GBM by focusing on recent preclinical and clinical studies that might help us to revisit the candidacy of COX-2 as a therapeutic target to manage this highly fatal brain tumor.

COX-2 pathways and tumors

COX, officially known as prostaglandin-endoperoxide synthase (*PTGS*), is the rate-limiting enzyme in the synthesis of bioactive molecules termed ‘prostanoids’; it converts membrane-released arachidonic acid into the intermediate prostaglandin G₂ (PGG₂), and then prostaglandin H₂ (PGH₂) by its hydroperoxidase activity. Therefore, COX is also known as prostaglandin G/H synthase. Short-lived PGH₂ is further transformed to five types of prostanoid[prostaglandin D₂ (PGD₂), PGE₂, PGF_{2α}, prostacyclin PGI₂, and thromboxane A₂ (TXA₂)] by tissue-specific prostanoid synthases (Figure 1) [13]. By activating a suite of G-protein-coupled receptors (GPCRs), prostanoids regulate a diversity of physiological and pathological events; for example: inflammatory and anaphylactic reactions by prostaglandins; platelet aggregation and vasoconstriction by thromboxane; and platelet inhibition and vasodilation by prostacyclin. Two GPCRs (DP1 and DP2) are bound and activated by PGD₂ and four by PGE₂ (EP1–EP4), whereas each of the other three prostanoids acts on a single receptor: FP via PGF_{2α}, IP via PGI₂, and TP via TXA₂ (Figure

1) [14,15]. The mammalian COX has two isozymes [COX-1 and COX-2 (or *PTGS1* and *PTGS2*)], both of which were discovered during the early 1990s. COX-3 was reported as a third form, but proved to be a truncated COX-1 lacking enzymatic activity in humans [16], although it might show some pathological activity in rodents, such as C6 glioblastoma development in rat models [17]. NSAIDs are nonselective COX inhibitors, such as aspirin, ibuprofen, and naproxen, whereas most commonly used selective COX-2 inhibitors (COXIBs) include celecoxib, rofecoxib, valdecoxib, and NS398 (Figure 2). The constitutive COX-1 present in most normal tissues maintains homeostatic prostanoids that are essential for a variety of physiological functions, whereas COX-2 expression is low at basal levels under normal conditions but is rapidly induced to mediate pathological events that are often associated with severe inflammatory processes in response to tissue injuries and other stimuli, such as lipopolysaccharides (LPS), excitotoxicity, cytokines, and growth factors [13,18–29]. Studies of the binding mechanisms of COXIBs demonstrated that they have two reversible steps involving both COX isozymes; however, their selectivity for COX-2 is mainly because of a third step that is irreversible and might be attributed to the presence of sulfone/sulfonamide that is able to fit into a side pocket of COX-2 (Figure 3) [30]. The pathophysiological significance of COX-2 renders the enzyme an appealing therapeutic target for many chronic inflammation-associated diseases, such as cancer (Figure 1) [13].

Growing evidence over the past decades indicates the involvement of COX-2 in the progression of a variety of tumors, including those of bladder [31], breast and ovary [32,33], colon [33–35], esophagus [36], head and neck [37], liver [38], lung [33], pancreas [39], prostate [33], skin [40], and brain [19,41]. COX-2 is often upregulated in tumor cells and tissues, accompanied by the elevated presence of prostanoids, particularly PGE₂. Epidemiological and experimental data suggest a positive correlation between the regular use of COX-2 inhibitor drugs and reduced rates of certain cancers and cancer-related deaths [35], although they are not US Food and Drug Administration (FDA)-approved for these indications. The molecular mechanisms whereby induced COX-2 promotes tumorigenesis are not fully understood; however, it is becoming clear that PGE₂ is the predominant COX-2-derived prostaglandin that facilitates tumor activities, including tumor cell adhesion, proliferation, migration, angiogenesis, immunosuppression, and metastasis [13,35,42]. An increasing body of evidence also suggests that COX-2 and PGE₂ have similar roles in the development and progression of malignant CNS tumors, such as glioblastomas [20]. Therefore, delineating the COX-2/PGE₂ signaling pathways in tumor cell activities holds the promise of new insights into the pathophysiology of GBM and might inspire novel therapeutic targets.

Expression of COX-2 and PGE₂ in glioblastomas

Most brain tumors, including astrocytoma, glioblastoma, meningioma, medulloblastoma, craniopharyngioma, oligodendroglioma, ependymoma and neurinoma, highly express COX-2, and most human malignant glioma cell lines show constitutively elevated levels of COX-2 [10,11,19,21,43–45]. COX-2 induction in human malignant brain tumors is also supported by the observation that the surgical removal of tumor tissues can reduce the PGE₂ levels in the plasma from patients with malignant but not benign brain tumors [46]. COX-2

expression in GBM cells and tissues appears to be regulated by several transcription factors, including nuclear factor- κ B (NF- κ B) and Sp1/Sp3 [43,47].

COX-2 has been reported to specifically accumulate in glioma cells, macrophages and microglia predominantly in areas surrounding tumor necrosis, whereas COX-1 is mainly present in the macrophages and microglia of the tumor parenchyma [48,49]. Geographically, the COX-2 induction in glioblastomas mostly occurs in the central regions of the tumors, followed by the peripheral regions, whereas COX-2 is poorly expressed or absent in the adjacent normal tissues. The regions of solid glioblastomas might provide a hypoxic microenvironment that could aggravate the malignant progression in human glioblastomas, and hypoxia can induce the COX-2 expression in human GBM-derived stem and progenitor cells, which is concomitant with an increase in invasive and migratory phenotypes of the cells [50].

COX-2 expression levels in glioblastomas are highly correlated with many aggressive aspects of the disease, such as rate of GBM cell proliferation [51], glioma grade [11], and poor prognosis [20], and so on. For instance, a study of surgical specimens from 66 patients with low- to high-grade astrocytomas suggested that the high COX-2 expression correlated with poor survival in human gliomas and this was particularly true in patients with GBM [12]. Another study of 43 patients with GBM showed that most glioblastoma tissues resected from these patients were COX-2 immunohistochemically positive. The median survival of patients with COX-2-negative glioblastomas was more than double that of those with COX-2-positive glioblastomas, and all patients who survived longer than 24 months had COX-2-negative lesions [52]. In addition, primary glioblastomas with high COX-2 expression levels in tumor cells had a shorter time to radiological recurrence than did those with fewer COX-2-positive tumor cells [49]. Therefore, independent of other variables, COX-2 expression in tumor cells has been proposed as a strong predictor of poor survival and the aggressiveness of gliomas [12].

Prostaglandin E synthase (PGES) is the terminal enzyme that directly synthesizes PGE₂ from COX-2-derived PGH₂ and has three isozymes: membrane-associated PGE synthase-1 (mPGES-1), mPGES-2, and cytosolic PGES (cPGES) (Figure 1). Similar to COX-2, all three PGES forms have been reported to be overexpressed in human glioma specimens in both low- and high-grade tumors, in total 94 tumor specimens, including astrocytoma, oligoastrocytoma, oligodendroglioma, and ependymoma, with higher expression in grade III tumors than in grade II tumors. Expression of COX-2, mPGES-1, and cPGES was found to be higher in recurrent tumors that required a second surgical removal than in tumors that were operated on only once [53]. Among the three isozymes, mPGES-1 is highly inducible and might be involved in apoptotic regulation in human glioblastomas [54].

COX-2 and glioblastoma proliferation, invasion, and angiogenesis

COX-2 induction in tumor cells contributes to tumor formation, growth, and metastasis in systemic cancers, predominantly via synthesizing PGE₂ that acts on its EP receptors to regulate cell proliferation, migration, apoptosis, and angiogenesis (Figure 1) [13,14,42,55].

Likewise, elevated COX-2–PGE₂ signaling in glioma cells and tissues might execute similar effects in the formation and progression of malignant gliomas in the brain.

The selective COX-2 inhibitor NS398 can reduce the proliferation and migration of the human GBM cell lines U-87MG and U-251MG, decrease the growth of 3D glioma spheroids, and promote apoptotic cells [11]. The rat C6 and human U138-MG glioma cell lines also showed decreased proliferation when treated with NS398 and other COX inhibitors (indomethacin, paracetamol, and sulindac) [56,57]. NS398 has also been demonstrated to inhibit the growth of human GBM A172 cells, in which COX-1 is highly expressed. The NS398-mediated antiproliferative effect in A172 cells can be diminished by adding external PGE₂ to the culture medium [58]. These findings together suggest that PGE₂ is a major driving force for COX-mediated A172 cell proliferation. Another COX-2 inhibitor, celecoxib, was reported to suppress the proliferation of several COX-2-positive GBM cell lines, including LN229, with higher potency than conventional nonselective NSAIDs (sulindac, flurbiprofen, and indomethacin) [44]. COX-2 is also essential for GBM cell migration and invasion. For instance, reactive oxygen species (ROS) promote the migration and invasion of human GBM U87 cells via extracellular signal-regulated kinases (ERKs)-dependent COX-2–PGE₂ activation [59]; the nuclear factor of activated T cells 1 (NFATC1) can also promote GBM U251 cell invasion through the induction of COX-2 [60].

Similar to other systemic tumors, as malignant gliomas, grow they typically require an augmented blood supply that involves angiogenesis. Antiangiogenic therapy has been proposed as a promising strategy to control GBM because malignant gliomas are typically highly vascular tumors. However, all Phase III clinical trials to date have failed to show any benefit of survival from antiangiogenic agents alone or in combination with traditional therapy [61]. COX-2 overexpression was found to strongly correlate with increased vascular endothelial growth factor (VEGF) and high microvessel density (MVD), and was substantially associated with a poor outcome. These findings for COX-2- and VEGF-related angiogenesis might suggest that COX-2 contributes to astrocytic tumorigenesis, at least in part, by promoting the formation of new vessels with prognostic implication [62]. Treatment of glioma cell lines with PGE₂ causes increased VEGF expression, indicating COX-2-mediated angiogenic effect is largely attributed to PGE₂ activity [47]. Given that COX-2 expression is related to MVD and the vascular pattern in glioblastomas, COX-2 inhibition might represent an antiangiogenic strategy for GBM [21]. Indeed, the selective COX-2 inhibitor rofecoxib, in combination with continuous low-dose scheduling of chemotherapeutic drugs, such as temozolomide, has been proposed as an antiangiogenic strategy to treat GBM [63].

COX-2 in immunosuppression and treatment resistance of GBM

COX-2 activity-driven inflammation in melanoma and other tumors has been proposed to nurture immunosuppressive microenvironments that allow tumor cells to escape immune scrutiny, a phenomenon that has been observed in both humans and experimental animals [64–66]. The ability to escape immune surveillance represents a large contributory factor to GBM formation and progression, and the lack of naïve effector T cells might explain the immune suppression and evasion mediated by GBM cells [67]. The hypoxic

microenvironment of solid GBM facilitates COX-2 induction [50], and COX-2 and its effector PGE₂ are well known to contribute to cellular immunosuppression in various cancers (Figure 1). Although the cellular and molecular mechanisms whereby COX-2 modifies the immune environment in tumors remain largely unknown, immunosuppressive cytokines that are regulated by COX-2 might have critical roles [67]. As a highly malignant brain tumor, GBM only requires a short time to relapse after standard treatment. Recurrent GBM is often extremely resistant to subsequent chemotherapy and radiotherapy, and the treatment resistance has been proposed to associate with the immunosuppression induced by the tumor [68]. Both chemotherapy and radiotherapy can induce COX-2 to synthesize PGE₂ in GBM cells to cause overexpression of immunosuppressive cytokines, such as interleukin 6 (IL-6), IL-10, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and to block T cell infiltration and proliferation. The conventional treatment-induced immunosuppression can be alleviated by the nonselective COX inhibitor diclofenac [69]. Likewise, incubation with the COX-2 inhibitor NS398 before but not after radiotherapy enhanced radiation-induced human GBM cell death [70]; celecoxib increased the radiosensitivity of GBM cells and, thus, reduced the clonogenic survival of irradiated cells [71]. All these findings together suggest that COX-2 and its PGE₂ product have essential roles in the overexpression of immunosuppressive cytokines in GBM cells and the subsequent suppression on the recruitment and proliferation of naïve effector immune cells, enabling GBM cells to modulate the local immune environment. Therefore, the combination of standard treatment with COX-2 inhibition or other immune therapies might provide a strategy to reduce the immune suppression and evasion observed in patients with GBM.

Preclinical studies

Higher COX-2 expression is clinically associated with more aggressive glioblastomas and has been proposed as a strong predictor for poor survival. The well-documented *in vitro* antiproliferative and antiinvasive effects from COX-2 inhibition on GBM cells motivated preclinical efforts to investigate the feasibility of controlling GBM formation and progression by various selective and nonselective COX-2 inhibitors. Celecoxib has been the front-runner in these efforts ever since (Figures 2 and 3). Long-term dosing (for 21 days) with celecoxib alone or in combination with the chemotherapy drug 13-*cis*-retinoic acid increased survival in mice with a xenograft of human GBM cells U-251MG, but not U-87MG [72]. COX-2 inhibition by celecoxib or aspirin has been shown to delay the development of glioma in mouse models via blocking systemic PGE₂ production and the associated development of myeloid-derived suppressor cells to limit the infiltration of cytotoxic T lymphocytes in the tumor microenvironment [73]. In addition, celecoxib has been reported to enhance the therapeutic effect of the chemotherapy drug temozolomide in a mouse GBM xenograft model, through its antagonism on cancer stem cells involving the Wnt/β-catenin pathway [74]. Likewise, treatment with celecoxib sufficiently enhanced the ionizing radiation (IR) effect in colony formation and increased IR-mediated apoptosis in CD133-positive glioblastomas, suggestive of the potential for celecoxib as a radiosensitizing drug for clinical use to control malignant gliomas [75].

Several signaling pathways have been demonstrated to be involved in the proliferation, survival, and invasion of glioblastomas, where COX-2 is highly expressed. For instance, the

COX-2 inhibitor celecoxib induces the apoptosis of human GBM cells and this might require the transcription factor NF- κ B [76]. It also has been reported that celecoxib inhibits human GBM cell viability by inducing DNA damage that results in p53-dependent G1 cell cycle arrest and autophagy, and p53 appears to sensitize GBM cells to celecoxib [77]. The inhibitor of DNA-binding protein 1 (Id1), a transcriptional regulator that has important roles in cell proliferation, differentiation, and senescence, has been identified as an effector of the p53-dependent DNA damage response pathway [78]. Indeed, overexpression of COX-2 or Id1 facilitated tumor progression in a mouse GBM model (Figure 4), whereas celecoxib and the genetic ablation of Id1 blocked COX-2 overactivation-mediated human GBM proliferation, migration, invasion, angiogenesis, and aggressiveness both *in vitro* and *in vivo* [45]. These findings suggest that COX-2 facilitates the malignant potential of human GBM at least partially through induction of Id1 and this is further supported by another recent study, in which COX-2-derived PGE₂ induced Id1 via EP4 receptor-dependent activation of mitogen-activated protein kinase (MAPK) signaling and another transcription factor, early growth response protein 1 (EGR-1) [79].

Human studies

Accumulating evidence from numerous epidemiological and experimental studies suggests that use of NSAIDs reduces risks for cancers of brain, breast and ovary, colon, head and neck, liver, lung, prostate, and skin. PGE₂, an essential inflammatory mediator synthesized by COX-2, has potential roles in tumorigenesis through direct mutagenesis, tumor growth and invasion, metastasis, immunosuppression, and angiogenesis. [13,42]. The tumor-promoting nature of COX-2 in various systemic cancers led to the hypothesis that COX-2 inhibition would reduce PGE₂ synthesis in glioblastomas and, therefore, prevent or modify tumor progression; this resulted in many case-control and cohort studies as well as clinical trials aimed at evaluating the therapeutic potential of COX inhibitors in human GBM.

A case-control study involving 236 patients with GBM and 401 control subjects in the San Francisco Bay Area from 1997 to 2000 suggested an inverse association between the use of aspirin, ibuprofen, naproxen, or other NSAIDs and the risk of GBM in adults [80]. Another study with 325 glioma cases and 600 frequency-matched controls in the metropolitan area of Houston (2001–2006) indicated that the regular use of NSAIDs was associated with a 33% reduction in the risk for glioma [81]. A case-control study on 517 cases and 400 population controls recruited at Columbia University Medical Center and the University of California, San Francisco from 2007 to 2010 was initiated to study the relation between NSAID use and the incidence of GBM. This study also revealed an inverse association between the duration for uptake of NSAIDs (aspirin, ibuprofen, and naproxen for more than 6 months) and the risk of glioma that supports an essential role for COX-2 in gliomagenesis [82].

However, in the National Institutes of Health (NIH)-American Association of Retired Persons (AARP) Diet and Health Study, which was initiated in 1995–1996 and registered 302 767 individuals, with 341 incident glioma cases and 264 GBM cases, no association was found between the regular use of aspirin/nonaspirin NSAIDs (for 1 year before the study) and the risk of glioma/glioblastoma as compared with controls [83]. Another large perspective study in Denmark was conducted from 2000 to 2009 with a total of 2688 glioma

cases and 18 848 population controls, in which each case was matched on age and sex to eight population controls. Despite no apparent association between use of aspirin or other NSAIDs (including COX-2 inhibitors) and the risk of glioma, there was a slight reduction in glioma risk with long-term use of low-dose aspirin (5 years) [84]. The inconsistency arising from these results could be largely attributed to the methodological limitations in case-control studies.

Assessing chronic drug use (5 years in some studies) in questionnaire/interview-based epidemiological studies is often challenging, and this might be particularly true in patients with GBM, whose neurocognitive functions and skills are often compromised by tumors [85,86]. Most of these population studies also lack sufficient statistical power partially in that GBM is relatively rare, although it is considered the most common malignant brain tumor. In addition, the effects of NSAIDs are unlikely to be the same across different histological subtypes of glioma, and only one case-control study, specifically focused on GBM, demonstrated an inverse association between use of NSAIDs and the disease [80]. Likewise, glioblastoma subtypes identified by the TCGA consortium demonstrate the heterogeneity and complexity of the disease [8,9], suggesting that different forms of GBM likely respond to COX-targeted treatment in different ways. Furthermore, most of these studies did not differentiate the effects from different COX inhibitors, which can have distinct pharmacodynamic and pharmacokinetic properties, as well as off-target activities, although all can inhibit the production of PGE₂ [15].

A Phase II clinical trial sponsored by M.D. Anderson Cancer Center and National Cancer Institute (NCI) (<https://clinicaltrials.gov/show/NCT00112502>), with 25 patients with GBM, demonstrated that the combination of 13-*cis*-retinoic acid with celecoxib failed to show improved efficacy compared with 13-*cis*-retinoic acid alone in the treatment of recurrent GBM [72,87]. In the same trial with 155 participants, temozolomide in combination with celecoxib did not establish a benefit compared with temozolomide alone [87,88]. Another Phase II clinical trial by the Sidney Kimmel Comprehensive Cancer Center and NCI studied the effectiveness of celecoxib in treating patients who were under treatment with antiepileptic drugs and radiation therapy for newly diagnosed GBM (<https://clinicaltrials.gov/show/NCT00068770>). Unfortunately, the trial was terminated because results from another study suggested that temozolomide and radiation improved GBM survival and, thus, raised an ethical concern over continuing the trial. More recently, a Phase II clinical trial was organized by M.D. Anderson Cancer Center to determine whether aspirin decreased the incidence of venous thromboembolism in patients with GBM (<https://clinicaltrials.gov/show/NCT00790452>). However, the trial also ended early because of a drug supply issue. There are a few currently active clinical trials for GBM involving NSAIDs and COXIBs and we await the results and their interpretation (<https://clinicaltrials.gov/>).

Concluding remarks and future perspectives

COX-2 is commonly and robustly upregulated in human GBM cells and tissues, and an increasing body of evidence from preclinical and clinical studies suggests that elevated COX-2 activity in turn contributes to GBM genesis and progression. The mutual

reinforcement between COX-2 and glioblastomas has been strongly supported by numerous *in vitro* experiments using various human GBM cell lines, and further validated in many preclinical studies based on GBM xenograft animals that were treated with NSAIDs and COXIBs. These COX inhibitors might be particularly useful in controlling the concomitant symptoms of GBM, such as seizures. Current seizure management in patients with glioblastomas mainly relies on antiepileptic drugs (AEDs), which cause adverse effects, such as bone marrow toxicity, skin reactions and CNS toxicity, that are more common in patients with brain tumors than in other forms of epilepsy [4]. In addition, tumor-related epilepsies are highly resistant to current AEDs partially because of the overexpression of efflux transporter proteins in glia and endothelial cells of the BBB [4,89,90]. COX-2 inhibition by several NSAIDs or COXIBs showed considerable antiepileptic and/or antiepileptogenic effects in several animal epilepsy models, [15,29,91], and prevented seizure-induced upregulation of endothelial P-glycoproteins in the BBB [92–94]. These findings position COX-2 as an ideal molecular target to control both GBM tumors and associated epileptic seizures.

However, the enthusiasm of evolving COX-2 as an appealing therapeutic target for GBM and the promise of repurposing NSAIDs and COXIBs to treat brain tumors have been complicated by mixed results from case-control studies and clinical trials discontinued because of unexpected reasons. The past decade has also witnessed a growing recognition of the undesired effects of selective COX-2 inhibitor drugs, which led to the withdrawal of two COXIBs [rofecoxib (Vioxx®) and valdecoxib (Bextra®)] from the US market [95]. Celecoxib (Celebrex®) is the only currently available drug that specifically targets COX-2 in the US, with FDA-mandated black box warning for its potential cardiovascular and gastrointestinal toxicities. In July 2015, the FDA once again strengthened the warning that nonaspirin NSAIDs increase the risk for heart attacks and strokes (www.fda.gov/Drugs/DrugSafety/ucm451800.htm). All these setbacks together make it unlikely that chronic COX-2 inhibition alone is a sustainable therapeutic strategy, even though short-term exposure might provide some benefits. However, COX-2 inhibitors should be considered as adjunct treatment owing to their effect on sensitization of GBM to traditional chemotherapy and radiotherapy [64–66,69–71,75]. In addition, nanocapsules could offer more-efficient strategies to deliver COX inhibitors to gliomas, as recent studies showed that nanocapsules increased the intratumoral bioavailability of indomethacin and enhanced its inhibition of the growth of implanted gliomas [6,57,96]. As another alternative strategy, certain diet-derived phytochemicals should also be considered to treat gliomas owing to their similar anti-inflammatory properties to those of COX inhibitors [97–100].

Finally, the lessons learnt from the COX-2 saga also suggest that manipulating the downstream signaling molecules in the COX-2 cascade might offer therapeutics that are more selective to pathway-specific components in the control of tumor proliferation, invasion, and metastasis [14,55,95]. Perhaps it is now time to shift our focus and effort from COX-2 itself to PGE₂ synthases and relevant EP receptors to seek novel molecular targets for GBM (Figure 1).

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Highlights

- Current treatment for glioblastoma fails to provide sufficient therapeutic outcomes.
- Overexpressed cyclooxygenase-2 (COX-2) contributes to the glioblastoma progression.
- COX-2 plays complex roles in glioma invasion, angiogenesis, immunosuppression, etc.
- COX-2 inhibitors sensitize glioblastomas to conventional chemo- and radio-therapies.
- COX-2 downstream signaling pathways might provide alternative targets for gliomas.

Figure 1.

Proposed cyclooxygenase-2 (COX-2) signaling pathways in glioblastoma multiforme (GBM). Responding to various stimuli, membrane-bound arachidonic acid is released and converted to the short-lived intermediate molecule prostaglandin H₂ (PGH₂) by COX, which has two isoforms: constitutive COX-1 and inducible COX-2. PGH₂ is then quickly converted to five prostanoids: prostaglandin D₂ (PGD₂), prostaglandin E₂ (PGE₂), PGF_{2α}, prostacyclin (PGI₂), and thromboxane A₂ (TXA₂), by tissue-specific prostanoid synthases. PGE₂ can be synthesized from PGH₂ by three isozymes [membrane-associated PGE synthase-1 (mPGES-1), mPGES-2, and cytosolic (c)PGES], among which mPGES-1 is traditionally considered inducible. Prostanoids exert their functions via acting on a suite of GPCRs: EP1, EP2, EP3, and EP4 for PGE₂; DP1 and DP2 for PGD₂; FP for PGF_{2α}; IP for PGI₂; and TP for TXA₂. COX-2-derived PGE₂ is proposed to mediate GBM cell proliferation and invasion, angiogenesis, immune suppression, and evasion via EP receptors. Only the major pathways are shown.

Figure 2. Chemical structures of nonselective cyclooxygenase (COX) inhibitors [nonsteroidal anti-inflammatory drugs (NSAIDs)] and selective COX-2 inhibitors (COXIBs) that have been widely tested in human glioblastoma multiforme (GBM) cell cultures, animal models, and human studies. Most of these COX inhibitors, except rofecoxib and NS398, are current US Food and Drug Administration (FDA)-approved drugs for other indications.

Figure 3.

Crystal structure of arachidonic acid (**a**) and celecoxib (**b**) in the active site of the human cyclooxygenase-2 (COX-2) dimer. The molecular docking for the interaction between COX-2 and small molecules was generated by Discovery Studio software. The X-ray crystal structure of human COX-2 was obtained from the Protein Data Bank (PDB, ID: 5IKT) of the Research Collaboratory for Structural Bioinformatics (RCSB). The key active site residues of COX-2 important for binding are depicted as lines with atoms colored (carbon, grey; hydrogen, white; nitrogen, blue; and oxygen, red), and the binding molecules (arachidonic acid and celecoxib) are shown as ball-and-stick model with atoms colored (carbon, grey; hydrogen, white; nitrogen, blue; oxygen, red; and sulfur, yellow). The selectivity of celecoxib for COX-2 is largely determined by its sulfonamide functional group (see the highlights), which can fit into a side pocket of COX-2.

Figure 4.

The development of intracranial tumors is enhanced by cyclooxygenase-2 (COX-2) and DNA-binding protein 1 (Id1). H&E staining was performed for coronal sections of mouse brains with xenograft tumors derived from either the human GBM cell line LN229 or COX-2/Id1-overexpressing cells. Brain tumors are denoted by arrows. Note that elevated COX-2 led to enhanced growth of intracranial tumors, which was recapitulated by Id1 overexpression in GBM cells. Scale bar = 2 mm. Reproduced, with permission, from [45].