Platelet-derived thromboxane A_2 induces cyclooxygenase-2 and epithelial-mesenchymal transition marker genes in U-87MG human glioblastoma cells

Sara Di Berardino,* Annalisa Contursi,* Stefania Tacconelli, Huzaifa Ali Ahfaz, Paola Patrignani

Department of Neuroscience, Imaging and Clinical Sciences, and Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University, Chieti, Italy

*These authors contributed equally.

ABSTRACT

Glioblastoma(GBM) is an aggressive, treatment-resistant brain tumor. Elevated platelet counts are associated with tumor growth, and platelets accumulate in GBM tumors. Our study found that cyclooxygenase(COX)-2 expression and prostaglandin(PG)E_2 biosynthesis increase during the formation of U-87MG GBM cell spheroids. The COX-2 inhibitors celecoxib and rofecoxib inhibited PGE_2 biosynthesis and reduced U-87MG spheroid growth. In cocultures of platelets with U-87MG spheroids, enhanced thromboxane(TX)B_2 was reduced by the selective exposure of platelets to aspirin, suggesting a platelet origin. In U-87MG cells, platelets increased the expression of COX-2 and epithelial-mesenchymal transition (EMT) marker genes. These effects were prevented by pretreating platelets with aspirin to inhibit TXA_2 biosynthesis or with a TXA_2 receptor antagonist. A TXA_2 mimetic induced both COX-2 and EMT markers in spheroids. Altogether, these findings indicate that platelet TXA_2 induces COX-2 and promotes EMT in U-87MG cells. Aspirin, by inhibiting platelet TXA_2, could contribute to reduced tumor growth and invasion in GBM.

Key words: platelets; glioblastoma; thromboxane A_2; aspirin; coxibs.

Corresponding author: Paola Patrignani, Systems Pharmacology and Translational Therapeutics Laboratory, Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University, Via Luigi Polacchi 11, 66100 Chieti, Italy.
E-mail: ppatrignani@unich.it

Contributions: PP, study concept; data analysis and interpretation; manuscript original drafting; statistical analyses; SDB, AC, data aquisition, analysis and interpretation; ST, HAA, contribution to manuscript writing and editing; ST, PP, funding. All the authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Funding: the authors acknowledge funding received by Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) [Fondi per la Ricerca Scientifica di Ateneo, (ex 60%)] to PP and ST. Moreover, it was funded by the European Union- European Social Fund-PON Research and Innovation 2014-2020 to AC.

Received: 9 January 2026.
Accepted: 22 February 2026.

Publisher's note: all claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Licensee PAGEPress, Italy
Bleeding, Thrombosis and Vascular Biology 2026; 5(s1):443
doi:10.4081/btvb.2026.443

Introduction

Glioblastoma (GBM) represents the most prevalent and aggressive type of malignant brain tumor in adults.[1] Current treatment options yield a survival rate of less than 5% beyond 5 years.[2] Epidemiological data indicate that regular intake of non-steroidal antiinflammatory drugs (NSAIDs) may be associated with a lower risk of GBM, potentially through the inhibition of cyclooxygenase-2 (COX-2) activity, thereby reducing prostaglandin(PG)E_2 biosynthesis. PGE_2 is a biologically active lipid mediator that plays a role in the development and progression of neoplastic diseases.[3] COX-2-dependent PGE_2 affects various GBM cellular functions, including proliferation, inhibition of programmed cell death (apoptosis), invasion into surrounding tissue, formation of new blood vessels (angiogenesis), and suppression of immune responses (immune escape), underscoring its potential as a therapeutic target.[4]

Aspirin is an NSAID that irreversibly inhibits the activity of COX-1 and COX-2 by acetylating a serine residue in the active site of the COX-isozymes, specifically at serine residues 529 of COX-1 and 516 of COX-2. At low doses, aspirin exerts its antithrombotic effects by selectively inhibiting platelet COX-1, thereby reducing thromboxane (TX) A_2 generation.[5] Aspirin use for 6 months or more is associated with a decreased risk of glioma. It is unknown whether aspirin acts through platelet inhibition or by directly affecting GBM cells. Elevated platelet counts in GBM patients also appear to be linked to tumor growth.[6]

Identifying new agents for tumor therapy and analyzing their effects requires models that closely resemble the patient's tumor. Traditional 2D cell cultures only partially mimic solid tumors and are inadequate for high-throughput screening (HTS).[7]

3D tumor spheroids are a better approach for HTS with glioma cell lines and ultra-low-attachment plates.[8]

Our study aimed to develop spheroids of human U-87MG cells, a widely used epithelial-like cell line derived from a human glioblastoma (brain cancer), commonly used in cancer research, neuroscience, and drug testing to model aggressive brain tumors.[9] COX-1 and COX-2 expression and prostanoid and other eicosanoid biosynthesis were evaluated. Additionally, the effects of selective COX-2 inhibitors (coxibs) such as rofecoxib and celecoxib on U-87MG cell spheroid growth were studied. An additional objective was to examine whether platelets cocultured with U-87MG cells under three-dimensional (3D, spheroid) or two-dimensional (2D) culture conditions influence the expression of COX-2 and epithelial-mesenchymal transition (EMT) gene markers. Our findings suggest that i) COX-2-dependent PGE_2 contributes to tumor growth in U-87MG cells, as evidenced by its decrease following treatment with selective COX-2 inhibitors; ii) platelet-derived TXA_2 induces COX-2 and promotes EMT in U-87MG cells, since the platelet effects are mitigated by selective inhibition of aspirin and a TXA_2 receptor antagonist. In conclusion, by inhibiting platelet TXA_2, aspirin may reduce tumor growth and invasiveness in GBM.

Materials and Methods

Culture of human glioblastoma cell line U-87MG and generation of 3D spheroids of U-87MG cells

U-87MG, a human glioblastoma cell line, was purchased by American Type Culture Collection (ATCC, Manassas, USA), and cultured in EMEM medium (Sigma Aldrich, Italy) containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S), and 2 mM of L-glutamine until confluence. To obtain 3D cultures of U-87MG cells, also known as spheroids, U-87MG cells were seeded in 24-well plates precoated with agarose as previously reported.[10]Briefly, to prepare a 1.5% (w/v) agarose-coated multiwell, agarose purified powder (Sigma-Aldrich, Italy) was dissolved in phosphate-buffered saline (PBS). After high-pressure sterilization, 0.5 ml of the agarose solution was added to each well, and the gelation occurred at room temperature. U-87MG cells were dissociated into single cells by trypsinization, seeded into agarose-coated wells at approximately $1x10^5$ in 0.5 mL of their complete medium, and incubated at 37°C with 5% CO_2 for 8 days. Images of spheroids were captured daily for up to 8 days using phase-contrast microscopy (Leica Microsystems), and spheroid diameters were measured using ImageJ. If the spheroid was almost round, the volume (mm^3) was calculated using the formula $1/6 \times \pi \times d^3 = 0.5236 \times d^3$ (d = diameter in mm). If the spheroid was ellipsoidal (like a rugby ball), the volume formula was $0.5236 \times L \times W^2$ [L = major diameter (mm); W = minor diameter (mm)].

Western blot analysis

Protein extraction of U-87MG spheroids was performed as previously described.[11,12] Protein expression was detected using the specific antibodies: COX-2 (Cayman, #160112, dilution 1:1000), COX-1 (Cayman, #160108, dilution 1:1000), anti-TXA_2 receptor(TP) (Cayman, #10004452, dilution 1:1000), and GAPDH (Santa Cruz Biotechnology, sc20357, dilution 1:1000, used as loading control). Optical density (OD) for different specific bands was quantified using Alliance 1D software (UVITEC).

Assessment of eicosanoid lipidomics of U-87MG spheroids

To investigate the effects of celecoxib and rofecoxib on eicosanoid biosynthesis, U-87MG spheroids, at 8 days of formation, were incubated with rofecoxib, celecoxib (at the final concentration of 10 µM), or vehicle (DMSO) at 37°C with 5% CO_2; then arachidonic acid (AA) (final concentration 10 µM) was added for a further 30 minutes (min), and supernatants were collected. In the supernatants, the levels of 12R/S-HETE, 15R/S-HETE, 5R/S-HETE, and 8R/S-HETE were assessed using liquid chromatography-mass spectrometry (LC-MS/MS).[13] Moreover, TXB_2 and PGE_2 levels were evaluated using specific immunoassays.[14] Values were reported as nM (evaluated in 0.5 mL of conditioned medium) corrected for the spheroid volume (mm^3).

Effects of Celecoxib and Rofecoxib on U-87MG spheroid formation

The U-87MG cells were dissociated into single cells and seeded in agarose-coated wells at a density of about $2x10^4$ cells per 0.5 ml of their complete medium (10% FBS, 1% P/S, and 1% L-glutamine) and incubated at 37°C with 5% CO_2 for up to 8 days without or with vehicle (DMSO) or 10 µM of celecoxib or rofecoxib (these compounds were added at day zero of spheroid formation). Spheroids were imaged by phase-contrast microscopy (Leica Microsystems), and spheroid diameters were measured using ImageJ; volumes (mm^3) were calculated as described above.

Coculture experiments with U-87MG cells in 2D or 3D conditions and isolated human platelets

U-87MG cells, cultured as previously described and dissociated into single-cell suspensions, were seeded at a density of $5x10^6$ cells into 12-well plates multiwell; after an overnight (~18 h after seeding), the culture medium was replaced with fresh EMEM medium (Sigma Aldrich) containing 0.5% FBS, 1% P/S, 2 mM of L-glutamine, and polymixin B sulfate 10 µg/ml (Sigma-Aldrich), to enable co-culture with platelets. Platelets were isolated from the concentrated buffy coat obtained by the Transfusion Centre (SS. Annunziata Hospital, Chieti, Italy) from healthy volunteers after signing informed consent, as previously described.[16] Then, 0.1 ml platelet suspension (containing $0.5x10^8$ cells) was added to U-87MG cells ($0.5x10^6$ cells) (U-87MG cells: platelets ratio was 1:100).[15,16]

In some experiments, U-87MG cell spheroids at 4 days of formation (as described above) were incubated with freshly isolated human platelets from the buffy coat, as previously described. 0.2 ml of platelet suspension (containing $1x10^7$ cells) was added to U-87MG spheroids ($1x10^5$ cells) (U-87MG cells: platelets ratio was 1:100).[15,16]

In a set of experiment, the isolated platelets were pretreated with Aspirin (acetylsalicylic acid, ASA) 100µM for 30 min (to acetylate COX-1 at serine 529 and irreversibly inhibit

COX-1 activity), and after extensive washing (to remove ASA) the acetylated platelets were added to U-87MG cell in adhesion or to spheroids at 4 days of formation; platelets *in vitro*, >95% inhibition of platelet TXB_2 biosynthesis requires an aspirin concentration of 100µM were also cultured alone. In vitro, an aspirin concentration of 100 µM, which exceeds circulating levels typically observed following low-dose administration, is required to achieve greater than 95% inhibition of platelet TXB_2 biosynthesis. This is because, *in vivo*, platelet COX-1 inhibition predominantly occurs presystemically- prior to first-pass metabolism -and does not directly correlate with drug levels in the systemic circulation. After 20 h, supernatants and cellular pellets were collected. Supernatants were centrifuged at 970g for 15 min at 4°C to remove platelets, then respun at 16,000 g for 40 min at 4°C, and the supernatants were collected. U-87MG spheroids cultured alone or with platelets were washed twice with PBS. Supernatants and cell pellets were then frozen in liquid nitrogen and stored at –80°C. In another set of experiments, U-87MG cells in adhesion or spheroids at 4 days of formation were pre-treated with SQ29,548 (10 µM) for 30 min before platelet addition. Finally, in some experiments, the U-87MG spheroids cultured for 4 days were incubated with U46619 (250 nM) for 4 h.

mRNA analysis

Total RNA was isolated from U-87MG cells cultured in 2D and 3D conditions using the Purelink RNA Mini Kit (Life Technologies). The levels of *PTGS2*, *TWIST1*, *VIMENTIN*, *SNAIL1*, and *GAPDH* mRNA were measured. Briefly, two µg of RNA were retrotranscribed using the iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA). Then, 100ng of cDNA was used in the reaction mixture, and amplification of *PTGS2*, *TWIST1*, *VIM*, *SNAIL1*, and *GAPDH* levels was performed using TaqMan gene expression assays (Hs00153133, Hs00361186, Hs00185584, Hs00195591, Hs99999905, respectively) on a 7900HT Real-Time PCR system (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

All data are reported as mean ± SD unless otherwise stated. Statistical analysis was performed using GraphPad Prism Software (version 10.0 for Mac, GraphPad, San Diego, CA, USA). Data were assessed for normality using the D'Agostino-Pearson test; the Student's *t*-test was used to compare the means of two independent groups, whereas one-way analysis of variance followed by Newman-Keuls *post-hoc* test was used to compare the means of more than two independent groups; *p*-values <0.05 were considered statistically significant.

Results

Culturing human U-87MG cells on a non-adhesive agarose gel layer prevents adhesion to the plate and promotes spontaneous self-assembly into 3D structures (spheroids) that mimic in vivo tissues. Spheroids were assembled within two days, and their diameter remained stable for up to 4 days; thereafter, they exhibited growth as a function of time (Figure 1A). COX-2 and COX-1 proteins were almost undetectable at 4 days by Western blot analysis. COX-2, but not COX-1, was induced at 8 days (Figure 1 B,C). The biosynthesis of targeted eicosanoids was assessed using LC-MS/MS and immunoassays at 8 days of culture. The biosynthesis of PGE_2 and TXB_2 served as indicators of COX isozyme activity, and the analysis of different hydroxyeicosatetraenoic acids (HETEs) in R and S configurations allowed the detection of enzymatic and non-enzymatic activities within AA metabolism, including 5-, 12-, and 15-lipoxygenases, as well as the HETEs produced by nonenzymatic oxidation. As depicted in Figure 1D, PGE_2 was the predominant eicosanoid generated by U-87MG cells in response to 10 µM of AA, accounting for approximately 88.2% (15±3.6 nM/mm^3) of the total eicosanoid production (17 nM/mm^3), while TXB_2 and all other eicosanoids were 10% (1.7±0.3 nM/mm^3) and 1.8%, respectively. Celecoxib and rofecoxib significantly reduced the biosynthesis of PGE_2 from spheroids of U-87MG human glioblastoma cells at day 8 by 83% and 73%, respectively, without significantly affecting the production of other eicosanoids (Figure 1D). This inhibitory effect of PGE_2 by the 2 coxibs was associated with a significant reduction in spheroid growth (Figure 1E).

U-87MG spheroids at 4 days cocultured with human platelets for a further 20 h caused platelet activation, as evidenced by increased TXB_2 levels (Figure 2A). TXB_2 generation was prevented by exposing platelets to aspirin (100 µM) before adding them to U-87MG cells, suggesting that TXB_2 generation in the coculture originated primarily from platelets. U-87MG spheroids cultured alone generated only low levels of TXA_2 (from endogenous AA), as measured by TXB_2 (Figure 2A), but they expressed the TXA_2 receptor TP at both 4 and 8 days (Figure 2 B,C). Platelets cocultured with U-87MG spheroids for 20h induced COX-2 expression in cancer spheroids (Figure 2D). TXA_2 increased COX-2 expression in U-87MG spheroids, as evidenced by the TXA_2 mimetic U46619 significantly increasing COX-2 gene expression in these spheroids (Figure 2E). Platelets and U46619 also enhanced TWIST1 expression, an EMT marker (Figure 2 F,G).[16]

To provide evidence that platelet TXA_2 triggers gene expression of COX-2 and EMT marker genes in cocultured U-87MG spheroids with platelets, we assessed the effects of aspirin exposure of platelets (to irreversibly inhibit TXA_2 generation) before adding them to the spheroids and of the TXA_2 receptor antagonist SQ 29,548. As shown in Figure 3 A-D, both aspirin and SQ 29,548 significantly prevented the induction of COX-2 and EMT genes (*TWIST1*, *SNAIL1*, *VIM*) by platelets. Similar results were observed in cocultures of platelets and U-87MG spheroids under adhesion 2D conditions (Figure 3 E-H).

Discussion

While the "Stupp protocol" (surgery, radiation, and temozolomide) remains the standard of care for GBM, several breakthrough therapies are currently under investigation, and some have received approval. Treatments for GBM are becoming increasingly personalized, leveraging recent advances in immunotherapy and innovative strategies to bypass the challenging blood-brain barrier.[17] Our study demonstrates that COX-2 is induced during U-87MG spheroid formation. COX-2 expression was associated with PGE_2 production, and inhibition of COX-2 by coxibs was associated with growth inhibition.

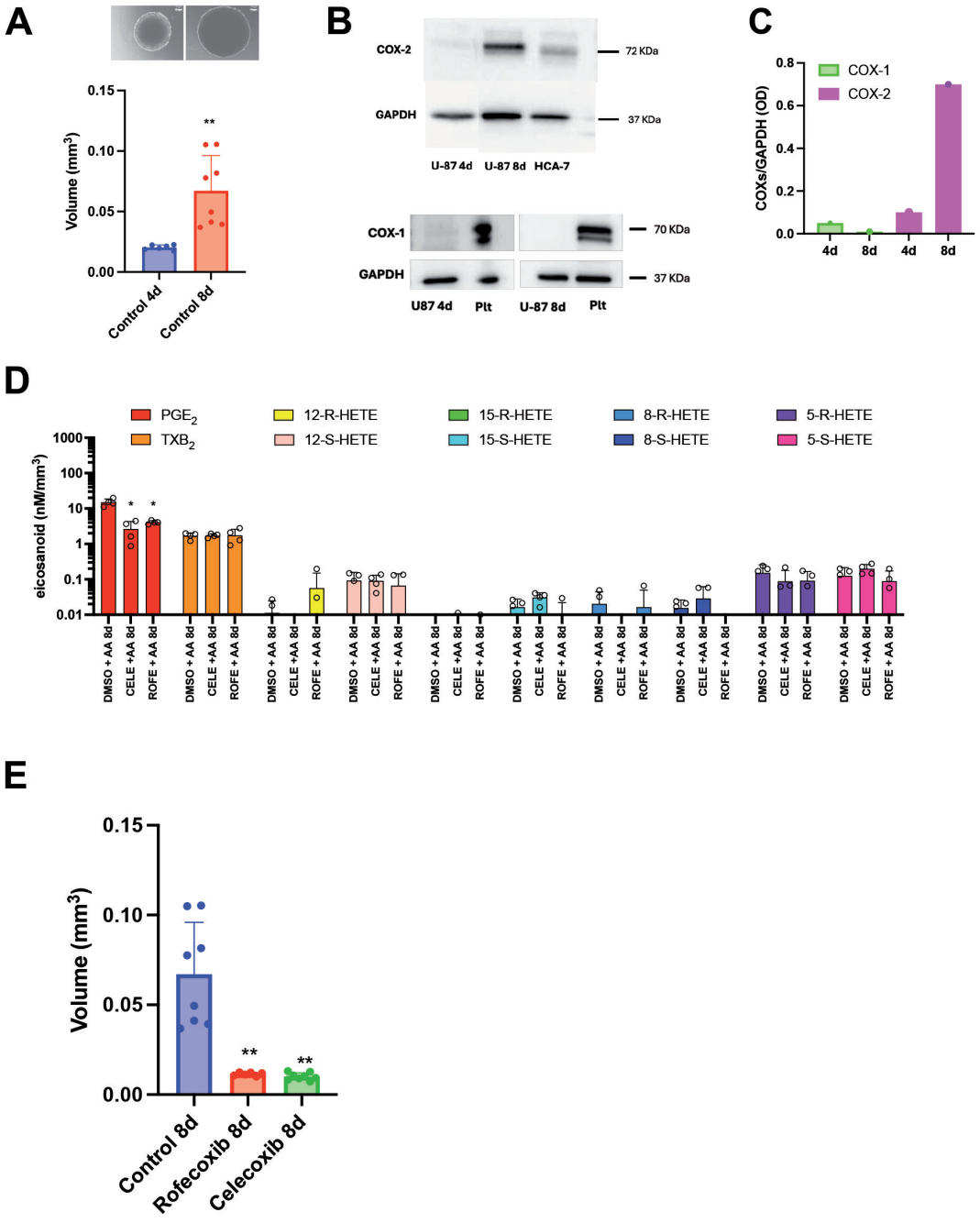

Figure 1. Characterization of spheroids of U-87MG cells and the effects of coxibs. U-87MG cells were dissociated into single cells, seeded into agarose-coated wells at approximately 1×10^5 cells, and incubated at 37°C with 5% CO_2 for up to 8 days. Images of the spheroids were captured at 4 and 8 days using phase-contrast microscopy (Leica Microsystems). The diameters of the spheroids were measured using ImageJ, and their volumes (mm^3) were calculated. **A)** U-87MG spheroid volume on days 4 and 8, along with representative images, is reported; data are presented as mean ± SD (n=6-8 separate experiments). Individual values are also displayed; **p<0.01 *vs* day 4; scale bars: 100 µm. **B)** The expression levels of COX-1, COX-2, and GAPDH (loading control) were assessed by Western blot. HCA-7 cells and platelets (Plt) served as positive controls for COX-2 and COX-1, respectively. **C)** Densitometric analysis of COX-2 protein levels relative to GAPDH loading control using optical density (OD). **D)** The effect of celecoxib and rofecoxib on the biosynthesis of eicosanoids by spheroids of U-87MG cells was examined. Spheroids on day 8 were incubated with rofecoxib (10 µM), celecoxib (10 µM), or vehicle (DMSO) at 37°C for 30 min, followed by the addition of AA (10 µM) for an additional 30 min. Subsequently, the supernatant was collected for eicosanoid quantification; values were reported as nM (evaluated in 0.5 mL) corrected for the spheroid volume (mm^3). The data are presented as mean+SD (n=4, separate experiments), and individual values are shown; *p<0.05 *vs* DMSO+AA. **E)** The effects of celecoxib and rofecoxib on the formation and growth of U-87MG spheroids are presented; after seeding U-87MG cells, either rofecoxib, celecoxib, or DMSO was added. The volume of the spheroids was subsequently measured on the eighth day; data are reported as mean+SD (n=6-9, separate experiments), with individual values also provided; **p<0.01 *vs* control.

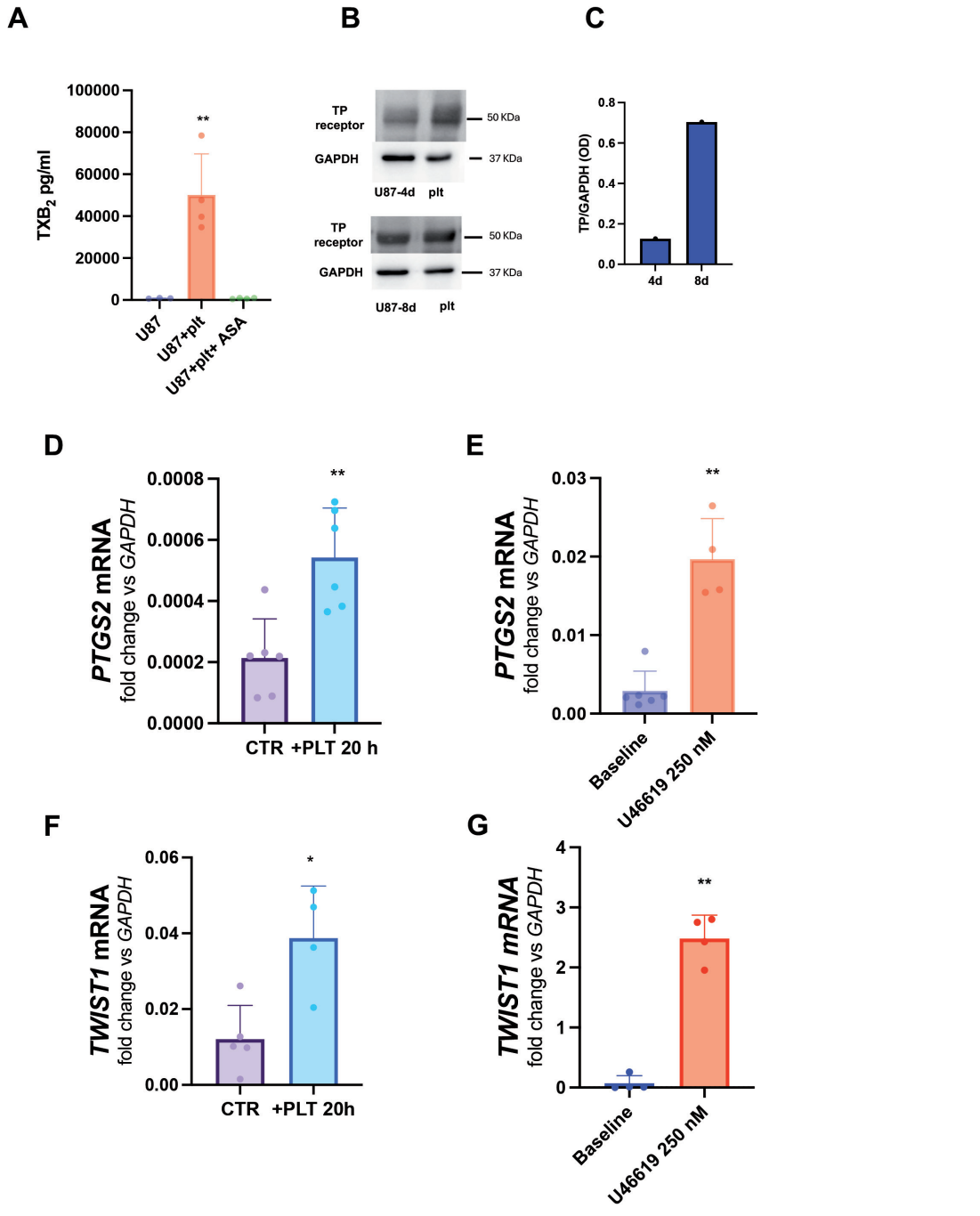

Figure 2. The interaction between platelets and U-87MG cell spheroids induces the expression of COX-2 and EMT marker genes. On day 4 of U-87MG spheroid cultures, the medium was replaced with fresh medium, and washed human platelets [pretreated with Aspirin (ASA) 100 µM for 30 min or its vehicle] were incubated for an additional 20h. Pellets were collected for qPCR analysis of *PTGS2 (*COX-2*), TWIST1*, and *GAPDH*; TXB_2 levels were measured in the supernatants using a specific immunoassay. In some experiments, U-87MG spheroids were incubated with vehicle (Baseline) or U46619 (a TXA_2 mimetic). **A)** TXB_2 levels measured in supernatants of U-87MG spheroids cultured alone or with platelets, pretreated or not with ASA; data are presented as mean+SD (n=4, separate experiments), and individual values are also displayed; $^{**}p<0.01$ *vs* other conditions. **(B)**The expression of TP receptors and GAPDH (loading control) was assessed by Western blot in platelets and U-87MG spheroids cultured alone on day 4 and day 8. **C)** Densitometric analysis of TP receptor protein levels relative to GAPDH loading control using optical density (OD). **D,F)** The gene expression of *PTGS2* (COX-2), *TWIST1,* and *GAPDH* (as an internal control for mRNA quantification) was evaluated in U-87MG spheroids cultured alone (CTR) or cocultured with platelets for 20 h by qPCR; data are presented as mean+SD (n=6, separate experiments), and individual values are also displayed; $^{**}p<0.01$, $^{*}p<0.05$ *vs* CTR. **E,G)** U-87MG spheroids were incubated with vehicle (Baseline) or U46619 (250nM) for 4 h, and *PTGS2* (COX-2), *TWIST1,* and *GAPDH* (as an internal control for mRNA quantification) were assessed by qPCR; data are presented as mean+SD (n=4-7, separate experiments), and individual values are also displayed; $^{**}p<0.01$ *vs* baseline.

Further studies should be conducted to evaluate the impact of COX-2 inhibitors on functional outcomes, including cell viability, proliferation, apoptosis, and invasion or migration. Furthermore, changes in spheroid integrity and the formation of a necrotic core should be assessed. PGE_2 is a prostanoid that contributes to tumor development through various mechanisms, including proliferation, invasion, and inhibition of apoptosis.[18] Furthermore, PGE_2 inhibits immune responses, facilitating immune evasion. Thus, combining a selective COX-2 inhibitor that inhibits PGE_2 biosynthesis could help mitigate resistance to immunotherapy in GBM. Despite promising preclinical findings, the use of COX-2 inhibitors is challenged by concerns about increased cardiovascular risk and thrombotic events arising from inhibition of the protective pathway involving vascular prostacyclin, which is produced by COX-2.[5]

Platelets accumulate in the distinctive "pseudopalisading" regions of GBM tumors, particularly around hypoxic and necrotic zones, where they interact with tumor cells and contribute to GBM growth. This localized accumulation underscores the potential role of crosstalk between platelets and cancer cells in the tumor's aggressive biology, suggesting that targeting platelet-tumor cell communication could offer viable therapeutic strategies.[19] We found that U-87MG cell spheroids produce low levels of TXA_2 but express TXA_2 receptors. Platelet

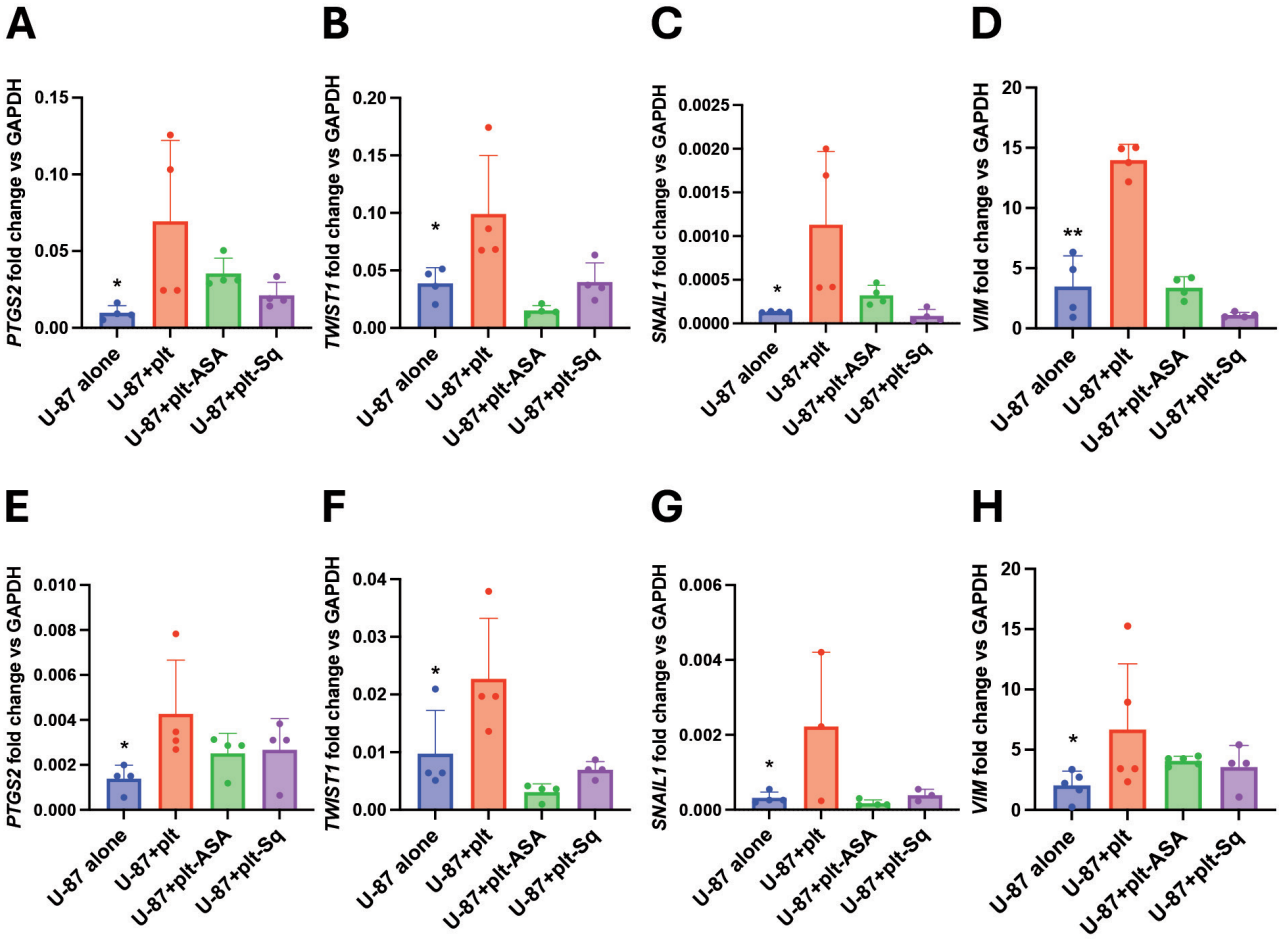

Figure 3. Effects of aspirin and a TXA_2 receptor antagonist on COX-2 and EMT marker gene expression in U-87MG cells cultured under 3D or 2D conditions with platelets. **A-D)** On day 4 of U-87MG spheroid cultures, the medium was replaced with fresh medium, and washed human platelets (plt) [pretreated with Aspirin (ASA) 100 µM for 30 min or its vehicle] were incubated for an additional 20 h. In some experiments, spheroids were incubated with SQ 29,548 (Sq, 10 µM) for 30 min prior to platelet addition, and the coculture was then maintained for 20 h. The gene expression of *PTGS2* (COX-2), *TWIST1, SNAIL1, VIM,* and *GAPDH* (as an internal control for mRNA quantification) was evaluated by qPCR in U-87MG spheroid cultures. Data are shown as mean + SD (n=4, separate experiments) with individual values shown; *p<0.05, **p<0.01 *vs* U-87+plt. **E-H)** U-87MG cells were cultured in 12 multiwell plates, alone or co-cultured with platelets (plt) that were not pre-treated or pre-treated with Aspirin (ASA). In another set of experiments, U-87MG cells were incubated with SQ 29,548 (Sq, 10 µM) for 30 min before platelet addition, and the coculture was then maintained for 20 h. The gene expression of *PTGS2* (COX-2), *TWIST1, SNAIL1, VIM,* and *GAPDH* (as an internal control for mRNA quantification) was evaluated by qPCR in U-87MG cells cultured alone or cocultured with platelets. Data are shown as mean + SD (n=3-5, separate experiments) with individual values shown; *p<0.05 *vs* U-87+plt.

interaction with U-87MG cell spheroids increases TXA_2 production, which induces COX-2 and EMT marker genes in U-87MG cells in both 3D and 2D cultures. These responses can be prevented by aspirin, a TXA_2 inhibitor, or a TXA_2 antagonist. However, the potential importance of the platelet TXA_2-COX-2 axis in GBM should be confirmed in patient-derived GBM stem-like cells or well-established lines such as LN229, U251, or T98G. In fact, a limitation of our study is that it used only U-87MG cells, which have genomic differences from primary GBM tumors and exhibit limited heterogeneity.

TXA_2, a potent lipid mediator, plays a significant role in platelet activation, vascular contraction, and inflammation. In the context of cancer, it promotes cell proliferation, migration, metastasis, and angiogenesis.[18] Recent studies have demonstrated that platelet-derived TXA_2 can evade immune detection of cancer cells by inhibiting T-cell functions. TXA_2 acts as a negative regulator of T-cell activation and proliferation by binding to the TP receptor on T cells, thereby initiating an immunosuppressive pathway dependent on the guanine nucleotide exchange factor ARHGEF1.[20] This pathway suppresses T-cell receptor (TCR)-driven signaling and effector functions. Aspirin inhibits platelet COX-1-dependent TXA_2 biosynthesis, thereby reactivating the immune response against cancer cells.

Confirmation of our findings on the role of platelet TXA_2 in promoting COX-2 and EMT across various GBM cell lines and glioblastoma primary cells isolated from patients will provide a rationale for in vivo studies in animal models and humans. These studies will evaluate whether aspirin, an antiplatelet drug that inhibits TXA_2 biosynthesis, or a TXA_2 receptor antagonist can reduce GBM growth, mitigate its aggressive and invasive nature, and overcome resistance to immunotherapy.

References

1. Thakkar JP, Dolecek TA, Horbinski C, et al. Epidemiologic and molecular prognostic review of glioblastoma. Cancer Epidemiol Biomarkers Prev 2014;23:1985-96.
2. Yin J, Kim SS, Choi E,et al. ARS2/MAGL signaling in glioblastoma stem cells promotes self-renewal and M2-like polarization of tumor-associated macrophages. Nat Commun 2020;11:2978.
3. Qiu J, Shi Z, Jiang J. Cyclooxygenase-2 in glioblastoma multiforme. Drug Discov Today 2017;22:148-56.
4. Zelenay S, van der Veen AG, Böttcher JP, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. Cell 2015;162:1257-70.
5. Patrignani P, Patrono C. Aspirin and cancer. J Am Coll Cardiol 2016;68:967-76.
6. Scheurer ME, El-Zein R, Thompson PA, et al. Long-term anti-inflammatory and antihistamine medication use and adult glioma risk. Cancer Epidemiol Biomarkers Pre. 2008;17: 1277-81.
7. Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol 2014;12:207-18.
8. Friedrich J, Seidel C, Ebner R, Kunz-Schughart LA. Spheroid-based drug screen: considerations and practical approach. Nat Protoc 2009;4:309-24.
9. Allen M, Bjerke M, Edlund H, et al. Origin of the U87MG glioma cell line: Good news and bad news. Sci Transl Med 2016; 31;8:354re3.
10. Li G, Liu Y, Yin H,et al. E-cadherin gene promoter hypermethylation may contribute to the risk of bladder cancer among Asian populations. Gene 2014;534:48-53.
11. Contursi A, Schiavone S, Dovizio M, et al. Platelets induce free and phospholipid-esterified 12-hydroxyeicosatetraenoic acid generation in colon cancer cells by delivering 12-lipoxygenase. J Lipid Res 2021;62:100109.
12. Patrignani P, Tacconelli S, Contursi A, et al. Optimizing aspirin dose for colorectal cancer patients through deep phenotyping using novel biomarkers of drug action. Front Pharmacol 2024;15:1362217.
13. Tacconelli S, Contursi A, Falcone L, et al. Characterization of cyclooxygenase-2 acetylation and prostanoid inhibition by aspirin in cellular systems. Biochem Pharmacol 2020;178: 114094.
14. Patrignani P, Tacconelli S, Piazuelo E, et al. Reappraisal of the clinical pharmacology of low-dose aspirin by comparing novel direct and traditional indirect biomarkers of drug action. J Thromb Haemost 2014;12:1320-30.
15. Dovizio M, Maier TJ, Alberti S, et al. Pharmacological inhibition of platelet-tumor cell cross-talk prevents platelet-induced overexpression of cyclooxygenase-2 in HT29 human colon carcinoma cells. Mol Pharmacol 2013;84:25-40.
16. Guillem-Llobat P, Dovizio M, Bruno A, et al. Aspirin prevents colorectal cancer metastasis in mice by splitting the crosstalk between platelets and tumor cells. Oncotarget 2016;7:32462-77.
17. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-96.
18. Wang D, Dubois RN. Eicosanoids and cancer. Nat Rev Cancer.2010;10:181-93.
19. Rong Y, Durden DL, Van Meir EG, Brat DJ. 'Pseudopalisading' necrosis in glioblastoma: a familiar morphologic feature that links vascular pathology, hypoxia, and angiogenesis. J Neuropathol Exp Neurol 2006 ;65:529-39.
20. Yang J, Yamashita-Kanemaru Y, Morris BI, et al. Aspirin prevents metastasis by limiting platelet TXA2 suppression of T cell immunity. Nature 2025;640:1052-61.