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Original article

Protease-activated receptor 1 mediated altered Ca^{+2} signaling in gliomasSukanya Tripathy^{a,f,1}, Bader O. Almutairi^b, Sanjay Singh^c, Atul Rawat^d, Durgesh Dubey^e,
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ABSTRACT

Thrombin-activated receptor-1 induces angiogenesis, cell proliferation, and invasion in tumors. The protein-activated receptor-1 (PAR1) is a G-protein coupled receptor that can be catalyzed by blood-derived serine proteases. Though Protease-activated receptor 1 plays an imperative role in coagulation and hemostasis, recent studies have found that PAR1 activation also affects the central nervous system (CNS) in addition to the vasculature. The present study focused on examining the prevalence of PAR1 in two human gliomas cell lines, D54 and U87, as well as the downstream signaling pathway. PAR1 was significantly more expressed in D54 cells than in U87 cells. An increase in PAR1 activity caused an increase in ERK1/2 activity, along with increased migration and invasion. SCH79797 (antagonist) significantly reduced the migration and invasion of the glioma cell lines. These cell lines' migration and invasion upon activator and inhibitor stimulation were related to PAR1 expression. In cells, Ca^{+2} concentration ($[Ca^{+2}]_i$) is altered by PAR1. Thrombin and TFLR both induced an increase in $[Ca^{+2}]_i$ that was inhibited by SCH79797 (PAR1 inhibitor). The study concludes that PAR1 signaling contributes to the progression of gliomas. PAR1 and its downstream signaling may act as potential therapeutic targets for the prevention of glioma formation.

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1. Introduction

Glioblastomas are the most common primary tumor within cranium, accounting for 80 percent of all malignant brain tumors. Malignant high-grade gliomas are anaplastic astrocytoma, oligodendroglioma (both WHO grade III), and gliomas multiforme (GBM; WHO grade IV), the most lethal of all glial neoplasms. (WHO grade II) Lower-grade (LG) tumors are slow-growing, infiltrating lesions that inexorably progress to a more aggressive tumor, commonly and pre-terminally a GBM. In the current treat-

ment strategies, such as surgical resection, radiation and chemotherapy, patient survival is only modestly improved, and patients suffer permanent deficits due to treatment-related toxicity (Gibson and Monje, 2012).

It has been reported that thrombin is expressed in a wide range of gliomas, including human gliomas and rat gliomas (Hua et al., 2005, 2008). Additionally, in rat gliomas, thrombin stimulates the release of VEGF from hypoxia-inducible factor-1* (HIF-1*) via mitogen-activated protein kinase (MAPK) pathways (Hua et al., 2003; Coughlin, 2000). Small molecular weight (MW 508) thrombin inhibitor argatroban treated rat F98 gliomas to reduce tumor mass, attenuate neurological deficits, and prolong survival time (Hua et al., 2003). Protease-activated receptors, PAR1, PAR-3, and PAR-4 (Coughlin, 2000), bind to thrombin and have been found on a variety of tumor cells, including rat gliomas (Coughlin, 2005). The PAR1 gene is activated during angiogenesis (Kaushal et al., 2006), tumor cell proliferation (Nierodzik et al., 1996), and invasion (Maragoudakis et al., 2000). Calcium signaling has been linked to a range of pathological conditions, including cancer. Sev-

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eral studies have found that calcium signals contribute to cancer cell proliferation, treatment resistance, and the spread of metastatic tumors. It has been shown that thrombin and PAR1-activating peptide (TFLLR) induces Ca²⁺ signals within rat brain microvascular cells. (Darmoul et al., 2004).

The present study aims at investigating the role of par1 (PAR1) in migration and invasion of gliomas. Further, it was also analyzed whether these receptors play a role in calcium influx which is amongst one of the important signaling molecules for the propagation of cancer cells.

2. Methods

2.1. Cell culture

Glioma cells D54 (RRID: CVCL_6748) and U87 (RRID: CVCL_EP65) were purchased from Thermo Fisher Scientific or NCCS, Pune. Cells implanted in DMEM containing 10% fetal bovine serum, 2 mM glutamine, 200 penicillin/ml, and 0.02 g/ml streptomycin under controlled humidity containing 5% CO₂. Cells were calculated using a hemocytometer.

2.2. RT-PCR analysis of PAR1 mRNA expression

A small Purelink RNA kit from invitrogen (Carlsbad, CA) was used to extract the total amount of RNA from D54 and U87 cells. 1 µg of RNA was loaded using the GeneAmp RT (Applied Biosystems) kit. Protease receptor primers are: forward 5' CCTATGAGACAGCCAGAATC 3' and reverse 5' GCTTCTTGACCTTCATCC 3' of protease receptor. GAPDH used as a leading controller 5' TTCAACGGCACAGTCAAGGC 3' The PCR cycle was maintained at 95 °C for 10 min, then at 94 °C for 30 sec, followed by anneal discharge at 51 °C (GAPDH), 55 °C (PAR1) for 90 s and 72 °C to extend for 30 s and 10 s.

2.3. Migration in D54 and U87 cells

The filters used were Transwell polycarbonate with 6 mm filters (Costar Corporation, Cambridge, MA). D54 and U87 glioma cells were starved to serum for 12 h and harvested with EDTA (1.5 mM). They were washed with PBS and returned to DMEM at 1.5 × 10⁶ cells/ml. An aliquot of 250 cells is placed at the top of the filter. SCH79797 was mixed with DMEM and added to the upper chamber (and cells), and 630 mL of DMEM with FBS was added to the lower chamber. For 5 h, the filters were also stored at 37 °C with 95% O₂ and 5% CO₂. The extra cells were immersed in PFA 4% for 5 min after being rubbed cells. 1% crystal violet was used to contaminate the extra cells.

2.4. Invasion assay in D54 and U87 cells

After harvesting semi-confluent suspensions of serum-stem cells, 2 lac cells per mL were established in DMEM. Cells were loaded into each matrigel-covered chamber. They were then placed back in the incubator for five hours. Lower cells are mixed with 4% paraformaldehyde (PFA) 5 min after rubbing the extra cells. Taint was carried out with 1percent violet in 0.2 M sodium borate buffer in the lower surface cells obtained. Untreated cells were used as controls.

2.5. Calcium flux assay

In order to analyze calcium permeability in glioma cells after activation and inhibition, a calcium flux assay was performed using Abcam Calcium Detection Assay Kit (ab102505 Abcam, Berlin). The

cells were seeded in 96 well plates for 24 h in serum deprived media treated with TFLLR and SCH79797. Cells were rinsed twice with 1 X sterile PBS the next day. Then 90 µL of the chromogenic reagent was added to each well containing the control and treated wells. 60 µL of Calcium Assay Buffer is added to each source. Plate was placed in the dark at room temperature for 10 min. Fraction is measured at 575 nm.

2.6. Statistical analysis

GraphPad Prism 8 used for statistical analysis. Results are presented as a standard ± SEM. Independent T-test and ANOVA were used to compare the methods. All statistical analyzes were performed with a mean value of p < 0.05.

3. Results

3.1. PAR1 receptor mRNA expression

In D54 and U87 cell lines, PAR1 expression was examined using RT-PCR. Evidently, the D54 cell line has a higher level of mRNA expression than the U87 cell line [p < 0.001; F (2:12) = 56.71] (Fig. 1a). Densitometric analysis was performed by Image Studio Lite Ver. 5.2 (Fig. 1b).

3.2. Active agent for PAR1 activation based on p-ERK

ERK^{1/2} signaling has been reported to be linked to protease receptor signaling downstream and is associated with pathogenesis of gliomas. In order to analyze the effect of protease activation on D54 and U87 cell lines, we used 30 µM TFLLR and different western blocks to release proteins not treated cells were defined as control group.. Continuous activation of p-ERK^{1/2} was detected in the D54 cell-line [p < 0.001; F (5, 32) = 864.0] but not in U87 cell-line [p < 0.01; F (5, 32) = 1059.0] when p-ERK^{1/2} activation decreased significantly (Fig. 2a). p-ERK^{1/2} in the D54 cell line redirect after 30 min and yet in the U87 cell-line signal intensity remained steadily lower after 30 min. (Fig. 2b). ERK1/2 is activated via improved phosphorylation and activation of the Protease receptor and may play a role in glioma pathogenesis.

3.3. PAR1 induced migration in D54 and U87 cells

In order to confirm PAR1 as a migration factor, we tested the effect of SCH79797 on TFLLR-based migration. The PAR1 inhibitor SCH79797 (3 mM) inhibited TFLLR-induced migration of D54 cell-line [F (2, 12) = 61.50, p < 0.001] (Fig. 3A), confirming role of PAR1 in the migration of D54 cell-line. U87 cells showed similar results to TFLLR-induced cells (Fig. 3B), where F (2, 12) = 8.45, p < 0.01].

3.4. PAR1 induced invasion in D54 and U87 cells

Thrombin, an endogenous ligand of PAR1, was used to confirm the activation and invasion of PAR1 by its agonist. D54 and U87 cell lines determined the concentration (EC₅₀) of thrombin. The D54 cells are activated by thrombin in low concentrations (0.1U/ml), but the U87 cells are activated only in high concentrations (1U/ml) (Fig. 4).

Thrombin was then studied for its effect on invasion. D54 cell-line invasion was significantly induced by thrombin [F (2, 12) = 88.48, p < 0.0001] (Fig. 5A) compared to U87 [F (2, 12) = 39.13, p > 0.05] (Fig. 5B).

SCH79797 was then used to evaluate the effect of par1 inhibition on TFLLR-induced invasion of cells. D54 cells were signifi-

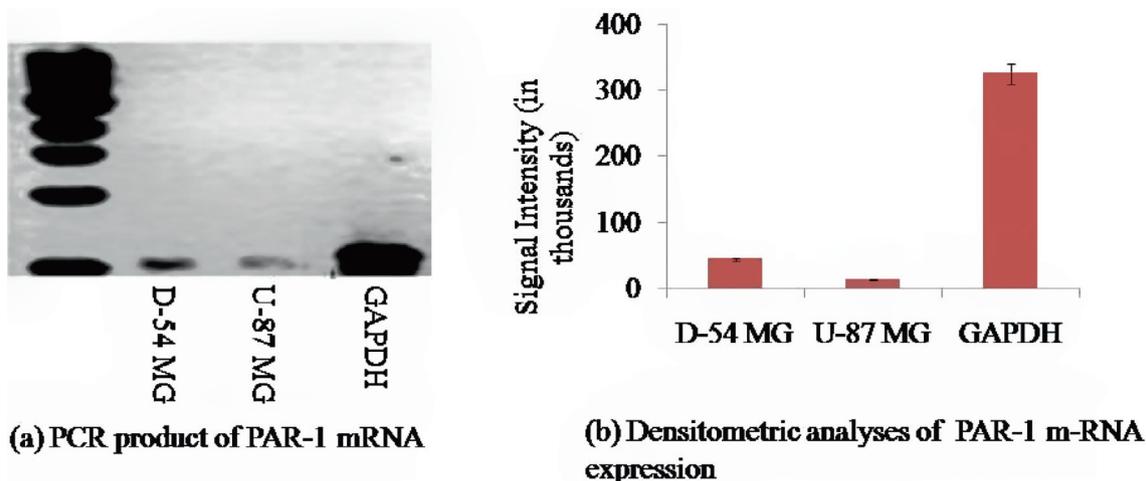


Fig. 1. (a) Represents the RT-PCR analysis of the PAR1 mRNA receptor. PCR is performed using specific sets of protease receptor and GAPDH. (b) Densitometric analysis of protease receptor m-RNA expression in D54 and U87 cell lines. m-RNA was genetically modified for GAPDH and expressed as descriptive ± SEM.

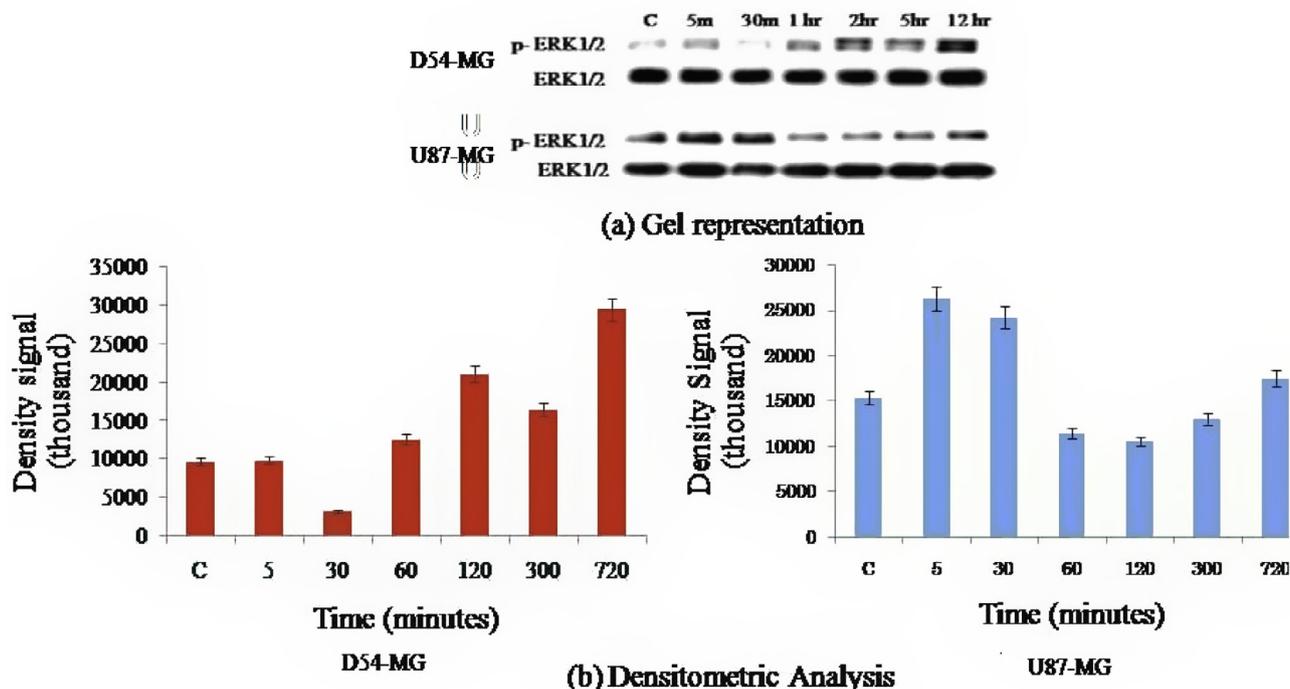


Fig. 2. (a) Cells stimulated by TFLLR and ERK 1/2 phosphorylation were tested for Western blot. (b) ERK 1/2 time interval activated by TFLLR (30 μM). Densitometric analysis results are defined as ± S.E.M. D54-MG cells showed continuous ERK 1/2 activity for >12 h while in U87 ERK 1/2 cells appeared to appear for only 1/2 hour.

cantly reduced in invasion with SCH79797 [F (2, 12) = 85.72, p > 0.0001] (Fig. 6A) and U87 cells were significantly decreased in invasion with SCH79797 [F (2, 12) = 8.984, p > 0.01] (Fig. 6B). The invasion of D54 cells across the matrigel was, however, highly significant as compared to U87 cells.

3.5. Calcium flux assay

Intracellular calcium concentration was analyzed using calcium flux assay. It was found that D54 cells show significant calcium influx [F (2,12) = 142.7] (Fig. 7A) when treated with TFLLR and SCH79797 while U87 show less significant calcium concentration [F (2,12) = 19.83] (Fig. 7B) when compared to the control group cells showed less concentration of intracellular calcium.

4. Discussion

The most malignant astrocytes are gliomas. Curing this disease is very difficult due to the pathogenic nature of the cells. Several studies have shown that the par1 gene facilitates the progression of gliomas and increases their severity (Xie et al., 2016). To investigate the association between the prevalence of protease receptor and its impact on the pathogenesis of gliomas we performed the present study. In two gliomas cell lines, the protease receptor expression was assessed. Using pharmacological interventions, we studied the role of this receptor in invasiveness and signaling. Cells from the D54 cell line express significantly more par1 mRNA than those from the U87. These findings suggest that the expression of protease-activated receptors on the cell surface is

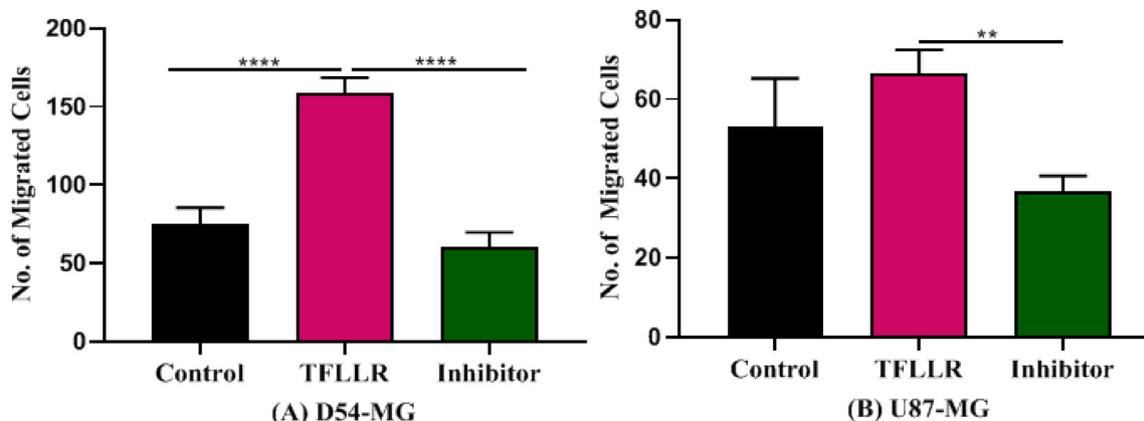


Fig. 3. Represents the effect of agonist and antagonist of protease receptor in migration of cells. The increase in migration of D54 cells by TFLLR was inhibited by protease inhibitor SCH79797 (3 μM). Number of migrated cells were decreased a concentration increased (n:5). (A) SCH79797 in the presence of TFLLR on the migration of cells D54 (B) SCH79797 in the presence of TFLLR on the migration of cells U87.

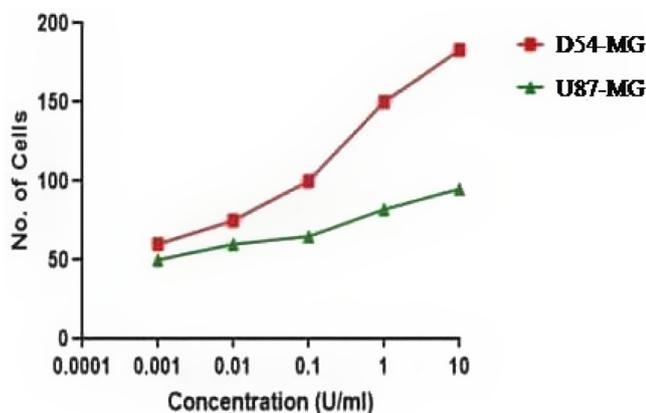


Fig. 4. D54 cells are activated at lower concentrations of thrombin, whereas U87 cells are activated at higher concentrations.

positively correlated with the expression of protease-activated receptors in messenger RNA. The expression of these receptors has been linked to an increase in malignancy in multiple studies (Maklad et al., 2019). Early in this century, studies linked ERK

1/2 signaling to par1 downstream signaling and to the pathogenesis of gliomas (Auvergne et al., 2016).

The activation of these receptors is then investigated to see if it alters the ERK12-dependent signaling pathway in these cell lines, which is linked to cell proliferation, migration, and invasion. Par1 activation causes astrocyte growth by activating MAPKs implicated in several pathways (Covic and Kuliopulos, 2018). TFLLR (a synthetic PAR1 activator) activated PAR1 in D54 and U87 cells, resulting in a rise in p-ERK 12. TFLLR administration resulted in continuously enhanced expression of p-ERK 1/2 in the D54 cell-line, but expression in the U87 cell-line reduced after 30 min, according to the study. The greater expression of PAR1 in the D54 cell line compared to the U87 cell line supported this conclusion. Recent research shows the protease-activated receptor and their ligand thrombin in glioma progression (Wojtukiewicz et al., 2015; Liu et al., 2017). A thrombin-binding protein is an endogenous PAR1 receptor ligand. By activating the receptor, it promotes the expansion and proliferation of gliomas (Zhu and Reiser, 2014). The central nervous system tumor cells of this study expressed PAR1, suggesting that thrombin or thrombin-like proteases (membrane attached or released by the tumor) are responsible for the auto-activation of these receptors. As a result of the activation of PAR1, thrombin causes neuroglial signaling to be amplified, inflam-

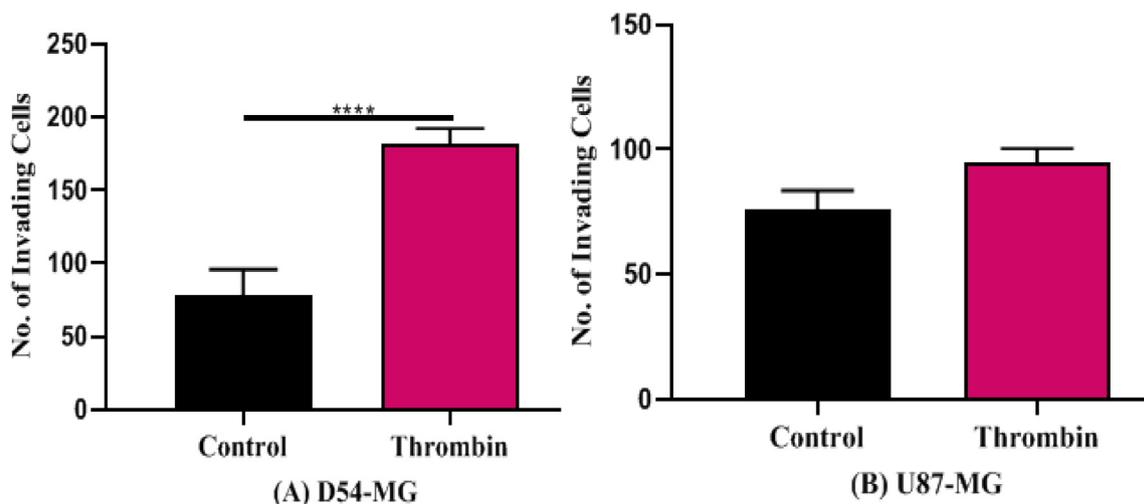


Fig. 5. Represents the EC50 of thrombin in D54 (A) and U87 (B) cell-line. D54 cells show increase at reduced concentrations of thrombin (0.1 U/ml) but U87 shows activation only at enhanced concentrations (1 U/ml) (n:5).

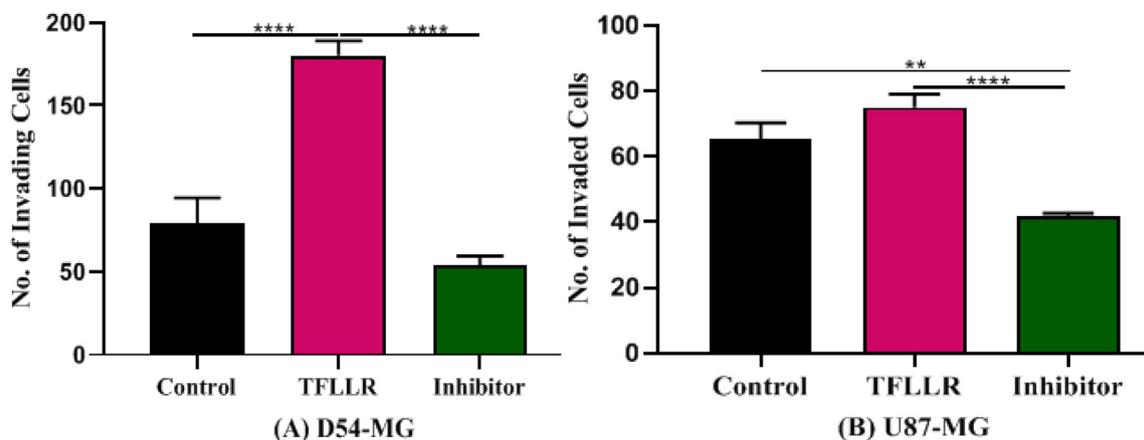


Fig. 6. Represents the effect of activator and inhibitor of par1 in invasion of cells. (A)Effect of antagonist SCH79797 in the presence of agonist TFLLR on the invasion of D54 (B) Effect of antagonist SCH79797 in the presence of agonist TFLLR on the invasion of U87.

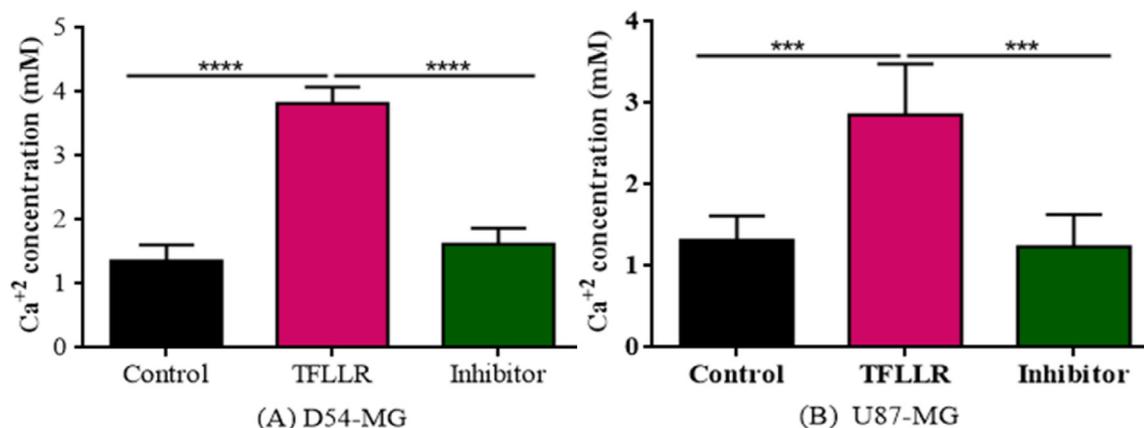


Fig. 7. Represents the effect of agonist and antagonist of PAR1 in influx of calcium. SCH79797 decreased the calcium flux. (A)Figure shows the effect of SCH79797 with TFLLR on the calcium flux of D54 cells. (B) Figure shows the effect of SCH79797 with TFLLR on the calcium flux of U87 cells.

mation, differentiation, proliferation, tumor-related brain edema, and malignancy in glial cells (Cheema et al., 2005). However, U87 cells migrated in a concentration-independent manner and increased only at a high concentration.

Next, we examined whether there was a link between the level of PAR1 receptor expression and the migration. Migration and invasion of D54 cells support the concentration, but it increases only at higher concentrations for U87 cells. Compared to U87 cells, D54 cells expressed more PAR1, suggesting a link between the PAR1 signaling pathway and pathogenicity. Several gliomas cell lines are shown to hold PAR1 receptors (Fan et al., 2005), putting them in danger for the event of gliomas cancer. Further studies have also linked angiogenesis with over-expression of PAR1 in Gliomas multiforme (Morrow et al., 2012).

As compared to U87 cells, D54 cells were significantly more likely to be induced to migrate and invade by TFLLR. As a result, PAR1 receptors are over-expressed within the D54 relative to the U87. The presence of PAR1 was confirmed by using SCH79797, a selected PAR1 inhibitor. The TFLLR-induced increase in migration and invasion of D54 was reduced significantly when the precise inhibitor SCH79797 was used. We confirm that PAR1 receptors are actively involved within the progression of Gliomas multiforme in rodents and in human tissues (Hua et al., 2008). These concomitant effects suggest the association of PAR1 expression with glioma migration. Previous studies have found that activated PAR1 was

not regulated in tumors (Chapman, 2013) by continued use of ERK1/2 and thus induced cellular pathogenesis.

Signaling mediated by calcium is one among the foremost important regulators of cellular activity. Calcium channels and pumps are abundantly expressed on the membranes of a broad range of cell types. Therefore, glutamate-like stimulants that activate calcium-channeling channels can activate Ca²⁺ infiltration into many cell types. It can cause 'wave-like' calcium stimulation at all tissue levels due to the subsequent increase in intracellular Ca²⁺ + proliferation (Itsekson-Hayosh et al., 2015). Calcium can be a signal molecule between cells that acts as a second messenger and transcripts think in many ways. Additionally, calcium channels and pumps cooperate extensively with other channels like voltage-gated proton, potassium, and chloride channels. The fate of cells is thus determined by calcium via an outsized network of signaling regulators. Enhanced metabolic changes, glial proliferation, angiogenesis, invasion, and migration, excessive calcium expression induced by the activity of the calcium channel may enhance the conversion and progression of glioma. (Pearson and Regad, 2017).

Thrombin and TFLLR activated cells are found to point out a dose-dependent rapid and transient [Ca²⁺] increase within the brain (Kuhn et al., 2014). This calcium influx was sensitive to the pathogenesis of gliomas. It's been reported that stimulation of PAR1 causes activation of PLC and Ca²⁺ release from the endoplasmic reticulum (López et al., 2019; Putney and Tomita, 2012). There has been evidence that PAR1 stimulation with TFLLR leads to a

huge influx of Ca²⁺ into the extracellular space via SOCCs (Kim et al., 2004; Chou et al., 2021; Lin et al., 2022; Yuan, 2000). When the cells were treated with PAR1 inhibitor SCH79797 the increase in calcium flux was decreased. It had been in line with the evidence that eliminating extrinsic Ca²⁺ or pre-incubating with SKF-96365, an inhibitor of SOCCs, prevented PAR1-AP-induced [Ca²⁺] rise. According to these results, activation of PAR1 and activator peptide TFLR results in calcium infection rather than inhibition. A secondary messenger, calcium (Ca²⁺), is involved in many cellular processes, including cell growth, apoptosis, differentiation, metabolism, contraction, neuronal plasticity, and transcription (Prevarskaya et al., 2014; Berridge et al., 2003; Monteith et al., 2017). Ca²⁺ signaling is involved in many physiological processes also as pathological processes, like cancer. Proliferation, angiogenesis, invasion, and metastasis are all Ca²⁺-dependent processes critical for cancer progression (Ali et al., 2016a,b, 2015; Ali and Ali, 2014). In recent years, Ca²⁺ signaling has also been shown to contribute to the expansion and tumorigenesis of cancers of the brain. Despite the bulk of those studies performed thus far on glioma, an increasing body of evidence indicates the importance of Ca²⁺ signaling in other sorts of brain cancer. Further, signaling of calcium in gliomas promoting growth and migration remains to be identified.

5. Conclusion

PAR1 is involved in the pathogenesis of gliomas, as discussed throughout the paper. Activator and inhibitor treatments of PAR1 demonstrate that the protein contributes to migration and invasion of gliomas. In addition, it is one of the most important mechanisms of calcium entry into cells. For the first time, the study shows that PAR1 is directly involved with glioma development through increased calcium influx.

Conflict of interest

None.

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References

- Ali, D., Ali, H., (2015). Assessment of DNA damage and cytotoxicity of palmatine on human skin epithelial carcinoma cells. 96:941–950. doi: 10.1080/02772248.2014.987510
- Ali, H., Dixit, S., Ali, D., et al., 2015. Isolation and evaluation of anticancer efficacy of stigmasterol in a mouse model of DMBA-induced skin carcinoma. *Drug Des. Devel. Ther.* 9, 2793–2800. <https://doi.org/10.2147/DDDT.S83514>.
- Ali, D., Verma, A., Pathak, A.K., et al., 2016. UVR-induced toxicity of sludge and polycyclic aromatic hydrocarbons on seed germination and seedling growth of *Triticum aestivum* L. 101080/0275754020161146711 32:446–459.
- Ali, H., Dixit, S., Ali, D., et al., 2016. Isolation and evaluation of biological efficacy of quercetin in human hepatic carcinoma cells. *Drug Des. Devel. Ther.* 10:155–162. doi: 10.2147/DDDT.S95275.
- Auvertgne, R., Wu, C., Connell, A., Au, S., Cornwell, A., Osipovitch, M., Benraiss, A., Dangelmajer, S., Guerrero-Cazares, H., Quinones-Hinojosa, A., Goldman, S.A., 2016. PAR1 inhibition suppresses the self-renewal and growth of A2B5-defined glioma progenitor cells and their derived gliomas in vivo. *Oncogene* 35 (29), 3817–3828.
- Berridge, M.J., Bootman, M.D., Roderick, H.L., 2003. Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.* 4, 517–529.
- Chapman, J., 2013. Coagulation in inflammatory diseases of the central nervous system. *Semin. Thromb. Hemost.* 39, 876–880.
- Cheema, T.A., Ward, C.E., Fisher, S.K., 2005. Subnanomolar concentrations of thrombin enhance the volume-sensitive efflux of taurine from human 1321N1 astrocytoma cells. *J. Pharmacol. Exp. Ther.* 315 (2), 755–763.

- Chou, H.-Y., Liu, L.-H., Chen, C.-Y., Lin, I.-F., Ali, D., Yueh-Luen Lee, A., David Wang, H.-M., 2021. Bifunctional mechanisms of autophagy and apoptosis regulations in melanoma from *Bacillus subtilis* natto fermentation extract. *Food Chem. Toxicol.* 150, 112020.
- Coughlin, S.R., 2000. Thrombin signalling and protease-activated receptors. [Review] [64 refs]. *Nature* 407 (6801), 258–264.
- Coughlin, S.R., 2005. Protease-activated receptors in hemostasis, thrombosis and vascular biology. *J. Thromb. Haemost.* 3 (8), 1800–1814.
- Covic, L., Kuliopulos, A., 2018. Protease-activated receptor 1 as therapeutic target in breast, lung, and ovarian cancer: Pepducin approach. *Int. J. Mol. Sci.* 19 (8), 2237.
- Darmoul, D., Gratio, V., Devaud, H., Peiretti, F., Laburthe, M., 2004. Activation of proteinase-activated receptor 1 promotes human colon cancer cell proliferation through epidermal growth factor receptor transactivation. *Mol. Cancer Res.* 2, 514–522.
- Fan, Y., Zhang, W., Mulholland, M., 2005. Thrombin and PAR1-AP increase proinflammatory cytokine expression in C6 cells. *J. Surg. Res.* 129, 196–201.
- Gibson, E., Monje, M., 2012. Effect of cancer therapy on neural stem cells: implications for cognitive function. *Curr. Opin. Oncol.* 24, 672–678.
- Hua, Y., Keep, R.F., Schallert, T., Hoff, J.T., Xi, G., 2003. A thrombin inhibitor reduces brain edema, glioma mass and neurological deficits in a rat glioma model. *Acta Neurochir. Suppl.* 86, 503–506.
- Hua, Y., Tang, L., Keep, R.F., Schallert, T., Fewel, M., Muraszko, K., Hoff, J.T., Xi, G., 2005. The role of thrombin in gliomas. *J. Thromb. Haemost.* 3, 1–7.
- Hua, Y., Vickers, T.A., Okunola, H.L., Bennett, C.F., Krainer, A.R., 2008. Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am. J. Hum. Genet.* 82 (4), 834–848.
- Hua, Y., Tang, L., Keep, R.F., Hoff, J.T., Heth, J., Xi, G., Muraszko, K.M., 2008. Thrombin enhances glioma growth. *Acta Neurochir. Suppl.* 102, 363–366.
- Itsekson-Hayosh, Z., Shavit-Stein, E., Last, D., et al., 2015. Thrombin activity and thrombin receptor in rat gliomas model: possible markers and targets for intervention? *J. Mol. Neurosci.* 56, 644–651.
- Kaushal, V., Kohli, M., Dennis, R.A., Siegel, E.R., Chiles, W.W., Mukunyadzi, P., 2006. Thrombin receptor expression is upregulated in prostate cancer. *Prostate* 66 (3), 273–282.
- Kim, Y.V., Di Cello, F., Hillaire, C.S., Kim, K.S., 2004. Differential Ca²⁺ signaling by thrombin and protease-activated receptor-1-activating peptide in human brain microvascular endothelial cells. *Am. J. Physiol. Cell Physiol.* 286 (1), C31–42.
- Kuhn, S.A., Martin, M., Brodhun, M., et al., 2014. Overexpression of protease-activated receptor type 1 (PAR1) in gliomas multiforme WHO IV cells and blood vessels revealed by NCAM-assisted gliomas border labeling. *Neurol. Res.* 36, 709–721.
- Lin, Y.-Y., Chen, C.-Y., Ma, D.-L., Leung, C.-H., Chang, C.-Y., Wang, H.-M., 2022. Cell-derived artificial nanovesicle as a drug delivery system for malignant melanoma treatment. *Biomed. Pharmacother.* 147, 112586.
- Liu, X., Yu, J., Song, S., Yue, X., Li, Q., 2017. Protease-activated receptor-1 (PAR1): A promising molecular target for cancer. *Oncotarget* 8 (63), 107334–107345.
- López, E., Lee-Rivera, I., Alvarez-Arce, A., López-Colomé, A.M., 2019. Thrombin induces Ca²⁺-dependent glutamate release from RPE cells mediated by PLC/PKC and reverse Na⁺/Ca²⁺ exchange. *Mol. Vis.* 25, 546.
- Maklad, A., Sharma, A., Azimi, I., 2019. Calcium signaling in brain cancers: roles and therapeutic targeting. *Cancers* 11 (2), 145.
- Maragoudakis, M.E., Tsopanoglou, N.E., Andriopoulou, P., Maragoudakis, M.-E., 2000. Effects of thrombin/thrombosis in angiogenesis and tumour progression. [Review] [24 refs]. *Matrix Biol.* 19 (4), 345–351.
- Monteith, G.R., Prevarskaya, N., Roberts-Thomson, S.J., 2017. The calcium-cancer signalling nexus. *Nat. Rev. Cancer* 17, 367–380.
- Morrow, D.A., Braunwald, E., Bonaca, M.P., Ameriso, S.F., Dalby, A.J., Fish, M.P., Fox, K.A.A., Lipka, L.J., Liu, X., Nicolau, J.C., Ophuis, A.J.O., Paolasso, E., Scirica, B.M., Spinar, J., Theroux, P., Wiviott, S.D., Strony, J., Murphy, S.A., 2012. Vorapaxar in the secondary prevention of atherothrombotic events. *N. Engl. J. Med.* 366 (15), 1404–1413.
- Nierodzick, M.L., Bain, R.M., Liu, L.X., Shivji, M., Takeshita, K., Karpatkin, S., 1996. Presence of the seven transmembrane thrombin receptor on human tumour cells: effect of activation on tumour adhesion to platelets and tumor tyrosine phosphorylation. *Br. J. Haematol.* 92, 452–457.
- Pearson, J.R.D., Regad, T., 2017. Targeting cellular pathways in gliomas multiforme. *Signal Transduct. Target Ther.*
- Prevarskaya, N., Ouadid-Ahidouch, H., Skryma, R., Shuba, Y., 2014. Remodelling of Ca²⁺ transport in cancer: How it contributes to cancer hallmarks? *Philos. Trans. R. Soc. B* 369 (1638), 20130097.
- Putney, J.W., Tomita, T., 2012. Phospholipase C signaling and calcium influx. *Adv. Biol. Regul.* 52 (1), 152–164.
- Wojtkiewicz, M.Z., Hempel, D., Sierko, E., Tucker, S.C., Honn, K.V., 2015. Protease-activated receptors (PARs)—biology and role in cancer invasion and metastasis. *Cancer Metast. Rev.* 34 (4), 775–796.
- Xie, Q., Bao, X., Chen, Z.H., Xu, Y., Keep, R.F., Muraszko, K.M., Hua, Y., 2016. Role of protease-activated receptor-1 in glioma growth. *Brain Edema* XVI, 355–360.
- Yuan, S.Y., 2000. Signal transduction pathways in enhanced microvascular permeability. *Microcirculation* 7, 395–403.
- Zhu, Z., Reiser, G., 2014. Signaling mechanism of par1 1-induced proliferation of astrocytes: Stabilization of hypoxia inducible factor-1 α triggers glucose metabolism and accumulation of cyclin D1. *Neurochem. Int.* 79, 20–32.