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Research Article

Biomarker-Guided Drug Repurposing and Molecular Validation of Angiotensin-2 Receptor Type-1 in Brain Tumor

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Abstract

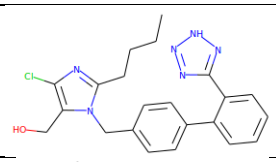
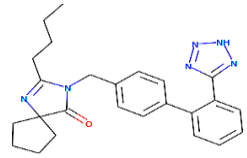
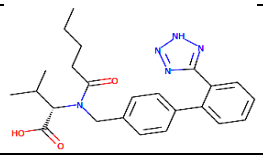
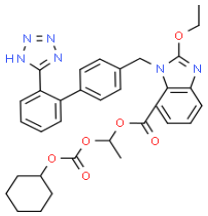
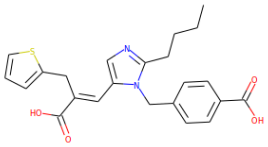
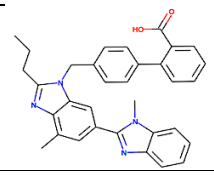
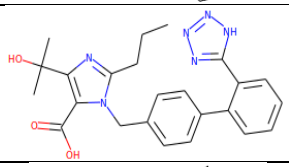
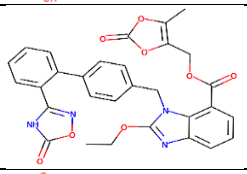
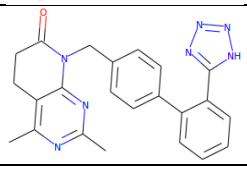
Glioma, the most frequent and malignant brain tumor, is one of the most lethal forms of cancer. Among a wide range of aberrantly expressed genes, Angiotensin-2 Receptor Type-1 (*AGTR1*) is reported to be up-regulated in glioma and associated with aggressive tumor traits and progression. The current study evaluates the anticancer potential of commonly used angiotensin receptor blocker (ARB) telmisartan. A total of eleven, Food and Drug Administration (FDA) approved ARBs were selected, and eight drugs were docked against the AGTR1 receptor. The antihypertensive drug with the highest docking score was selected for *in vitro* experimentation. Half maximal inhibitory concentration was determined and subsequently applied to patient-derived glioma cell lines. Quantitative gene expression post-drug treatment was determined through real-time polymerase chain reaction (PCR). *In vitro* growth inhibitory assays revealed that telmisartan at a dose of 45±0.06 µM was able to inhibit 50% of the cell population in malignant glioma U87 cell lines. The PCR results show that *AGTR1* expression in the untreated sample was high, as evidenced by $2^{-\Delta\Delta Ct}$ values (342.4, 138.5, 1467.3) for sample IK148-Glioblastma multiform (GBM), IK163-low grade glioma (LGG), and IK231-Medulloblastoma (MDB), respectively. The comparison was made with positive control U-87 cell lines. Following the drug treatment, an increase in the cycle threshold (Ct) mean value for *AGTR1* was observed, coinciding with a decrease in mean fold changes of 0.06±0.8, 0.5±2.0, and 0.02±0.02 for GBM, LGG, and MDB samples, respectively. Based on the findings of this study, it can be concluded that telmisartan exhibits successful inhibitory effects against *AGTR1* expression in glioma cell lines.

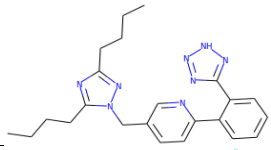
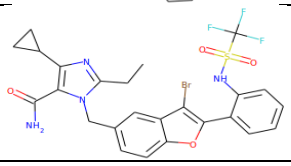
Keywords: Brain tumors, glioma, patient-derived cells, *AGTR1*, telmisartan

1. Introduction

Acute Cancer remains a major global health burden with a high mortality rate. Its treatment options are limited to surgery, radiation therapy, and chemotherapy, all of which come with significant long-term side effects. Among

Table 1: Showing the generic and brand names with 2D structures and drug bank accession numbers of the used ARBs for molecular docking (Wishart et al. 2018)

S/no	Generic Name	2D structure	Brand Name	Drug bank Accession Number
1	Losartan		Cozaar, Hyzaar	DB00678
2	Irbesartan		Avalide, Avapro, Ifirmacombi, Karvea, Karvezide	DB01029
3	Valsartan		Dafiro, Diovan, Diovan Hct, Entresto, Exforge, Exforge Hct	DB00177
4	Candesartan cilexetil		Atacand, Atacand Hct	DB00796
5	Eprosartan		Teveten, Teveten HCT	DB00876
6	Telmisartan		Actelsar Hct, Micardis, Micardis-hct, Pritor, Twynsta	DB00966
7	Olmesartan		Azor, Benicar, Benicar Hct, Olmetec, Olmetec Plus, Tribenzor	DB00275
8	Azilsartan medoxomil		Edarbi, Edarbyclor	DB08822
9	Tasosartan		Experimental	DB01349

10	Forasartan		Experimental	DB01342
11	Saprisartan		Experimental	DB01347

different types of cancer, gliomas are more prevalent and extremely fatal (Altieri et al. 2014), with Glioblastoma Multiforme (GBM) grade IV being the most aggressive with the highest reported fatality rate. Despite the availability of treatment options, the survival rate is low (average survival below 15 months) (Redzic et al. 2016). The limited treatment options and their expensive nature necessitate looking for alternates that are not only budget-friendly but also produce desirable outcomes (Kalinina et al. 2011). Recent advances in the field of genetics have greatly contributed to the search for alternative options. Several biomarkers are currently being studied for prognostic, therapeutic, and diagnostic purposes. Among these biomarkers, *AGTR1* can also be targeted for therapeutic purposes. *AGTR1* is one of the most extensively studied genes in the renin-angiotensin system (RAS), playing a significant part in maintaining renal-mediated water homeostasis, electrolyte, and blood pressure (Jiang et al. 2021). It is a G protein-coupled receptor found in organs such as the kidney, liver, heart, and brain (Kaschina, Steckelings, and Unger 2018). The *AGTR1* gene is more than 55kb long and is found at position 3q21-25 (Antonellis et al. 2002). It has five exons where the initial four codes for 5' UTR (Untranslated Region), and the fifth exon is the coding one (Guo et al. 1994) and has 359 amino acids (De Gasparo et al. 2000). When bound to angiotensin II, *AGTR1* stimulates angiogenesis, cell proliferation, and growth (Egami et al. 2003). At the same time, the opposite effects are observed when angiotensin II binds to another similar AGTR1 receptor (Goto et al. 2002). While *AGTR1* is normally expressed in multiple organs, its overexpression is associated with cancer formation (Takeda and Kondo 2001).

The angiotensin peptides and renin cannot pass into the brain because of the blood-brain barrier (BBB) (Fei et al. 1982; Ganten et al. 1976). The brain region outside of the BBB, such as circumventricular organs in the brain, is influenced by peripheral RAS (McKinley et al. 1990). Due to BBB, most brain regions are unable to access peripheral RAS, necessitating the production of cerebral RAS by the brain. The main source of locally produced angiotensin comes from the central RAS, which also influences the medulla and hypothalamus (Wright and Harding 2013; Lenkei et al. 1997; Bodiga and Bodiga 2013). Various neural cells in the brain, such as oligodendrocytes, astrocytes, and neurons, as well as components of basal ganglia, microglia of the cortex, and hippocampus, have been found to express AGTR1 receptors (Labandeira-Garcia et al. 2017).

On the other hand, the expression of *AGTR1* in a specific brain region may be aberrantly expressed depending on an individual's physiological state. Dehydration, stress, hypertension, chloride deficiency, and salt deficiency all affect the expression pattern of *AGTR1* in distinct parts of the brain (Barth and Gerstberger 1999; Charron et al. 2002; Saavedra et al. 1986; Sandberg, Ji, and Catt 1994; Ray et al. 1990). Moreover, the elevated expression of

ATGR1 is accountable for the increased release of specific cytokines, including transforming growth factor (α&β), vascular endothelial growth factor, platelet-derived growth factor, epidermal growth factor, basic fibroblast growth factor, and tumor necrosis factor-α, which are causative agents of tumor angiogenesis (Wang et al. 1999). Henceforth, the present study aims to determine the expression pattern of *AGTR1* in glioma tissue and patients-derived cell lines, followed by its inhibition through commercially available AGRT1 inhibitors for drug repurposing.

Table 2: Primer used for AGTR1 gene detection in tissues and cell lines of glioma patients.

Primer	Primer Sequence	Target Gene	RefSeq Accession	Tm	Amplicon Size (bp)
Forward Primer	CAGCGTCAGTTTCAACCTGTACG	*AGTR1*	NM_004835	60	95-140
Reverse Primer	GCAGGTGACTTTGGCTACAAGC				

2. Materials & Methods

2.1. AGTR1 Structure Download and Active Sites Prediction

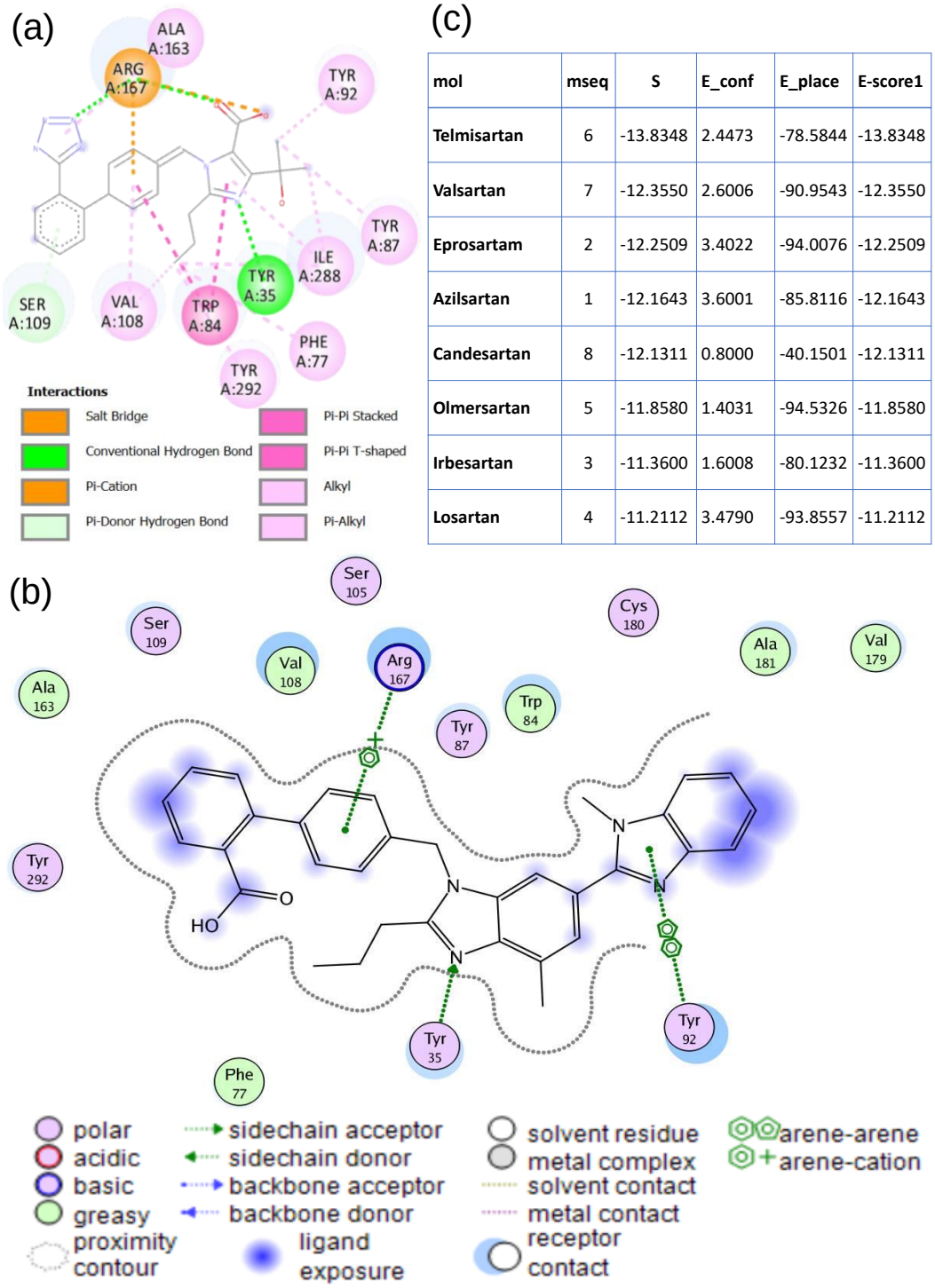
The three-dimensional structure of AGTR1 protein (ID 4ZUD) was retrieved from Protein Data Bank (PDB) website (https://www.rcsb.org/). This structure is in a complex with inverse agonist olmesartan at 2.8Å. The active sites were determined from existing literature (Zhang et al. 2015).

2.2. Molecular Docking

The structure of protein 4ZUD was prepared for docking in molecular operating environment-MOE. Solvent and ligands other than olmesartan were removed, and "protonate 3D" command in MOE was applied to add hydrogen and partial charges to the protein structure. This prepared structure was then saved. Eleven notable FDA-approved ARBs were selected through a drug bank (Wishart et al. 2018) for molecular docking to select a drug candidate with high binding energy to be repurposed as a potential anticancer drug. Their 2D structures and information is provided (**table 1**). The drugs that were in the experimental phase were excluded. The 3D structures of selected ARBs were downloaded from the ZINC database (Irwin et al. 2020) in SDF format, along with their specific identifier, and a library was created in MOE software. Drugs having the same identifier were excluded. Energy minimization and protonate 3D steps were executed in MOE software, and prepared drugs were saved in '.mdb' file format. The prepared drug structure of ARBs was docked against the active site residues of prepared protein by using the MOE tool. The docking study results were noted, and the top-scoring drug was selected to be included in the wet lab validation studies.

2.3. Tissue samples Processing

This study was approved by the ethical approval committee of Khyber Medical University and Hayatabad Medical Hospital, Peshawar, Pakistan, from where the samples were collected. Non-probability convenient sampling techniques were employed to collect three tumor samples based on histopathology reports. The specimens collected from the hospital were delivered within 15 minutes of tumor removal in a dry form and stored in cold condition (4°C). The tumor tissue was subjected to culturing protocol per established laboratory protocols (Khan et al. 2017). Each tumor was assigned a unique identifier and then divided into pieces. Part of it was stored in RNA*later* (Sigma-Aldrich, USA) solution, and the rest was cultured to generate cell lines.



mol	mseq	S	E_conf	E_place	E-score1
Telmisartan	6	-13.8348	2.4473	-78.5844	-13.8348
Valsartan	7	-12.3550	2.6006	-90.9543	-12.3550
Eprosartam	2	-12.2509	3.4022	-94.0076	-12.2509
Azilsartan	1	-12.1643	3.6001	-85.8116	-12.1643
Candesartan	8	-12.1311	0.8000	-40.1501	-12.1311
Olmersartan	5	-11.8580	1.4031	-94.5326	-11.8580
Irbesartan	3	-11.3600	1.6008	-80.1232	-11.3600
Losartan	4	-11.2112	3.4790	-93.8557	-11.2112

Figure 1: Molecular docking result of selected ARBs with AGTRI receptors. (a) 2D interaction of olm with AGTR1 structure (4ZUD), (b) 2D interaction of telmisartan with AGTR1 structure (4ZUD) (c) molecular docking result of different ARBs against AGTR1

2.4. Receptor Detection in Tissue Samples

The primer sequence for the *AGTR1* gene was obtained from GeneCards (https://www.genecards.org/) (Safran et al. 2021) (**table 2**). Total RNA extraction from tissue saved in RNA*later* was performed via trizol (Rio et al.

2010). *AGTR1* gene was amplified using primers through PCR, and its expression was analyzed with reference to the housekeeping gene Glyceraldehyde 3- Phosphate Dehydrogenase *(GAPDH).*

2.5. Patient Derived Primary Cell Lines Generation

Soon after surgical resection, the tumor samples were processed for cell line generation. The tissue was washed with phosphate buffer saline (PBS) (Sigma-Aldrich, USA) and minced in Hank's Balanced Salt Solution (HBSS) (Gibco, USA). The extracellular matrix (ECM) was disrupted with the help of 0.4 mg/m collagenase type 1a (125 U/mg) (Gibco, USA) to dissociate tissues into individual cells as described (Khan et al. 2017). The cell suspension was incubated in shaking condition for 10-15 minutes and centrifuged at 600 RCF (Relative Centrifugal Field) for 3 minutes. The cells' palate was resuspended in the growth medium of Dulbecco's Modified Eagle's Medium and Ham's F-12 Nutrient Mixture (DMEM-F12) media containing 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin (Gibco, USA). The cells were incubated at a fixed temperature of 37°C with a 5% CO_2 supply, humidified environment was provided. Cell growth was checked regularly, and cells were monitored for infection prevention. The expression of *AGTR1* in prepared cell lines was compared against positive control U-87 and reported as $2^{-\Delta\Delta Ct}$. U-87 is a malignant glioma cell line, and the target gene expression in this cell line is reported to be high (Rouillard et al. 2016).

2.6. *In Vitro* Growth Inhibitory Assay

The glioblastoma cell line U87-MG was generously provided by Norah Defamie from Norah Defamie Pôle Biologie Santé, Université de Poitiers, France. A total of 10,000 U-87 cells were seeded in 96 wells plates along with 90% DMEM-F12 media, 10% FBS, and 1% antibiotics. The plate was then incubated for 24 to 48 hours in an incubator. Upon confluency, telmisartan was applied in a serial dilution as 200µM, 100µM, 50µM, 25µM, 12.5µM, 6.25µM, and 0µM (untreated negative control) and incubated again for 48 hours to determine the maximal half inhibitory concentration of each drug-dose. After incubation, the cells were fixed in 4% paraformaldehyde solution and stained with 0.1% crystal violet (CV), as previously described (Viola et al. 2022). The CV stains the adherent cells as the dead cells are washed out. Hence, the amount of dye retained in a well is directly proportional to the number of cells that survived in the well. The CV stain was washed out, and 200µL of glacial acetic acid (Merck, Germany) was added per well. The absorbance was measured at 630nm, and analysis was done on GraphPad Prism v.8.4.3.

2.7. Anticancer Activity of Telmisartan by Optimized IC_{50} Dose

The patient-derived primary cell lines were subcultured to initial passages and transferred to 6 well plates. Upon confluence, 45 µM of telmisartan was applied, and the plate was incubated for 48 hours. After incubation, the drug was washed out with PBS, and total RNA was extracted by trizol reagent, as described (Rio et al. 2010). To ensure stability, the total RNA was immediately reverse transcribed using the OneScript cDNA Synthesis kit (Applied Biological Materials (abm) Inc, Canada, cat#G234) following the manufacturer's instruction. *AGTR1* gene in the sample and control was then amplified in real-time PCR, with GAPDH expression used as an experimental control.

3. Results

3.1. Three Amino Acids Anchoring the ARBs

It has been reported that tyrosine-35 (Y-35), tryptophan-84 (W-84), and arginine-167 (R-167)

Table 3: This table shows the demographic data of the samples recruited for the *in vitro* analysis of telmisartan on patient derived primary cell. The data was generated by clinical and histopathological record obtained post-surgery. P/R represents primary or recurrent tumor.

Identifier	Age	Gender	Residency	Sub Classification	Grade	P/R	Tumor Site	Familial
IK-148	11	Female	Kohat	Glioblastoma	IV	P	Parietal Lobe	No
IK-163	12	Male	Abbaspur	Low grade glioma	I	P	Left occipital region	No
IK-231	6	Female	Peshawar	Medulloblastoma	IV	P	Ventricular Lesion	No

bind ARBs to the active site of the receptor protein (Zhang et al. 2015). The 2D interaction of drug-target in MOE has visually confirmed this interaction. The analysis of the 2D interaction of olmesartan with *AGTR1* shows that this drug forms three hydrogen bonds, among the other type of interactions, within the active site (**figure 1a**). A molecular docking study of ARBs with the three active site residues Y-35, W-84, and R-167 reveals that telmisartan has the highest docking score of -13.8348 (**figure 1b and 1c**). The 2D interaction also confirms that telmisartan forms tight interactions with the AGTR1 receptor.

3.2. *AGTR1* High Expression in Glioma Tissue Samples

The tissue pieces were snap-frozen, and ground and total RNA was extracted via the trizol method to determine the expression pattern of *AGTR1* in the glioma sample. The PCR results revealed that all three glioma tissue samples (IK148-GBM, IK 63-LGG, and IK231-MDB) exhibited higher expression of *AGTR1* across all three samples (**figure 2a**). The results were compared to the expression profile of experimental control GAPDH. Among the samples, IK231-MDB tissue showed the highest expression of AGTR1 (21.26±0.2), followed by IK148-GBM (23.98±0.8) and IK163-LGG (26.48±1.8). After confirming elevated levels of *AGTR1* expression in tissue samples, the selected samples were further processed to generate patients-derived primary cell lines.

3.3. Patient-Derived Primary Cell Lines Developed from Three Glioma Tissue Samples

A total of three tumor samples were subjected to cell line generation protocol. All three samples initiated primary glioma cell lines successfully. During the initial week, the visible number of attached cells was low, and most of the cells lacked distinct morphologies, indicating their adaptation to the *in vitro* growth condition. In the second week, the cell number increased, occupying a greater proportion of space. Subsequently, in the third week, substantial cell growth was observed. Notably, multiple cell colonies were prominent in IK148-GBM and IK163-LGG, while IK231-MDB showed uniform distribution of cells (**figure 2a**).

3.4. Determination of Telmisartan IC_{50} Value Using Commercial Glioblastoma Cell Lines

To quantify the IC_{50} values of telmisartan on fast-growing commercial GBM cell lines, a dose range of 0-200µM was applied. The experimental compound exhibited a dose-dependent inhibition of cell viability. At 200µM, the cells' morphology is greatly affected, and after CV staining, the number of attached cells was almost negligible. When the dose was reduced to 100µM, the cell morphology remained somewhat the same, with only a few stained cells observed. Further decreasing the concentration showed that the number of surviving cells increased, and the cells also started to change their shape from circular to

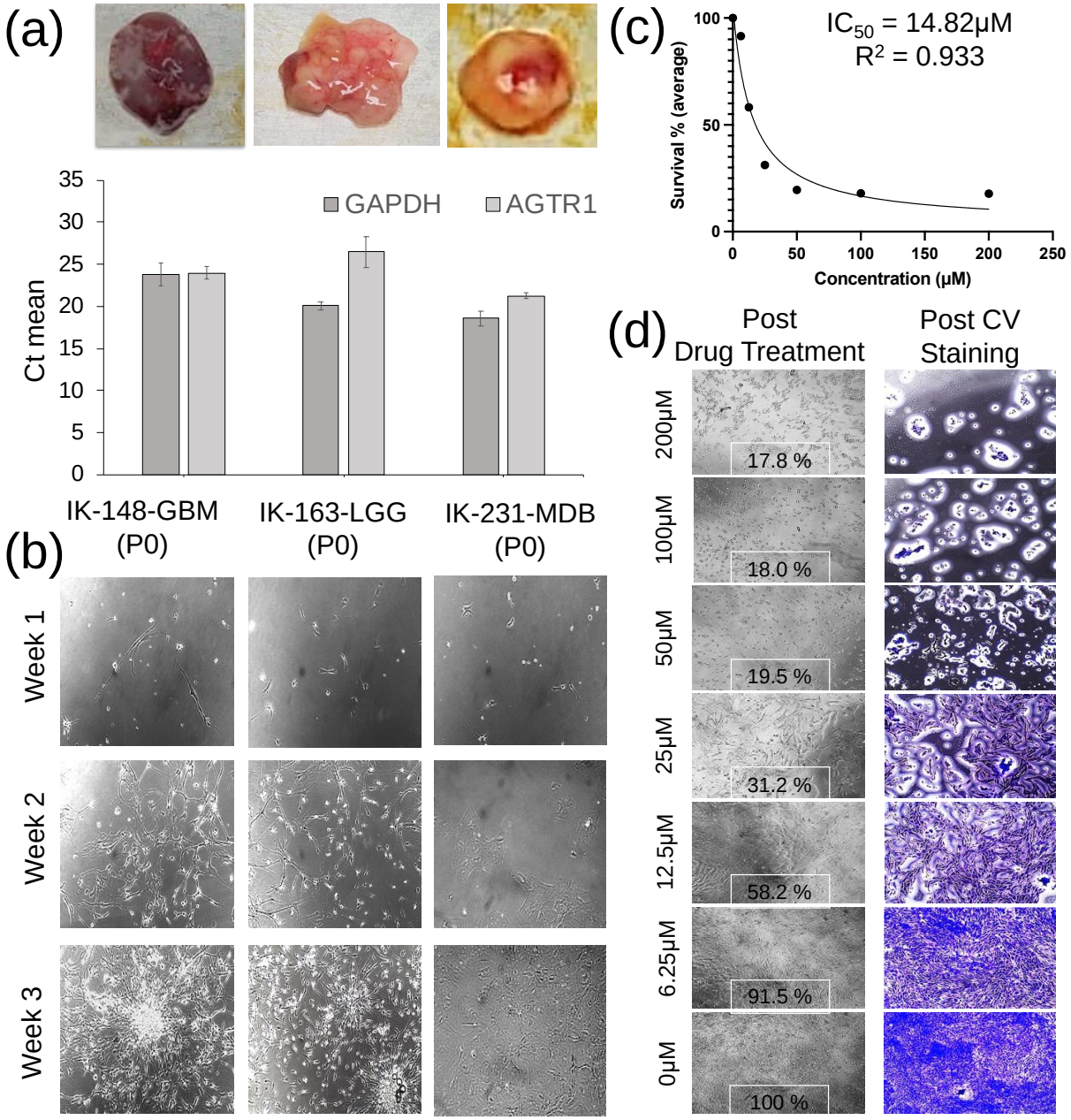


Figure 2: In Vitro assays to the anticancer potential of telmisartan against primary glioma cell lines. (a) Represents the three selected tissue pieces and their corresponding gene expression profile by quantitative real time PCR. The expression pattern of AGTR1 was compared with experimental control GAPDH. (b) Patient derived primary cell lines established from fresh dry tumor samples and maintained in growth medium until desired confluency is achieved. Photographs were captured after 1st, 2nd, and 3rd week of culturing. (c) Corresponding cell viability graphs computed on GraphPad prism. Percentage of cell survived is presented on the vertical axis and drug concentration on the horizontal axis. (d) Cells photographs recorded before and after staining with crystal violet dye post-treatment with drug for 48 hours

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elongated (**figure 2d**). Upon analyzing the result using graph pad prism v.8.4.3, it was observed that 14.82μM±0.06 could inhibit cell growth up to 50% **(figure 2c)**. The triplicate of this dose (45 μM±0.06) was then selected for subsequent experiments.

3.5. Telmisartan Inhibiting Cell Survivability in Glioma Cell Lines

When studying the anticancer potential of telmisartan on commercial cell lines, it was observed that telmisartan inhibited cancerous cell growth in a dose-dependent manner (Figure 2d). Telmisartan was then administered to the primary cell lines based on the IC_{50} value obtained from the commercial malignant glioma cell line. Additionally, it was noted that the dose-response differed with changing glioma subtypes.

3.6. Inhibition of AGTR-1 Observed in Highly Aggressive GBM

Telmisartan greatly affected the cell viability in highly aggressive GBM tumors when applied in half maximal growth inhibitory concentration. Since AGTR1 is reported to be linked with the aggressive nature of glioma, one can speculate that this receptor will be present abundantly. Since GBM is highly aggressive and malignant in nature, the tissue samples showed high expression of *AGTR1*. With the inhibition of AGTR1 with the receptor blocker drug telmisartan, the number of cells left alive was reduced. Comparisons were made between treated and non-treated cell lines with GAPDH as experimental control (**figure 3b**). It was observed that the expression of *GAPDH* and *AGTR1* in untreated cells were 29.21±0.9 and 34.84±0.07, respectively (**figure 3b**). Once treated with telmisartan, this Ct mean value of both the *GAPDH* and *AGTR1* were changed to 28.79±1.09 and 38.46±5.2, respectively (**figure 3b**). When the fold change of *AGTR1* compared to *GAPDH* was computed, a reduction of 0.06±0.8 was observed (**figure 3b**), indicating only 6% *of AGTR1* genes were expressed post-treatment.

3.7. Telmisartan Inhibited AGTR1 in Low-Grade Glioma (LGG) to a Lesser Extent

The same experiment was performed on an LGG sample, where cells were allowed to grow to confluency, treated in a dose of 45±0.06μM telmisartan, and incubated for 48 hours (**figure 3c**). Subsequently, total RNA extraction, cDNA synthesis, and PCR amplification were performed. Quantitative analysis of the PCR revealed a decrease in the fold change from 1 (control) to 0.5±2.0 (**figure 3c**) with the mean Ct value of 27.65±0.4 (*GAPDH*) and 34.58±1 (*AGTR1*) in the untreated cells (**figure 3c**). The Ct mean values observed after drug treatment corresponds to 28.18±0.8 (*GAPDH*) and 36.06±1.8 (*AGTR1*), as shown in (**figure 3c**). Although telmisartan reduced the expression of *AGTR1* in low-grade glioma to about 48.5%, this inhibition was lower than IK148-GBM. This indicates that the expression of *AGTR1* is lower than the aggressive tumor type. Therefore, its inhibition was also lower as compared to IK148-GBM. This is consistent with our gene expression pattern obtained from tissue samples (**figure 3c**).

3.8. Higher Inhibition Rate Observed in Medulloblastoma

A different type of glioma sample, medulloblastoma, was selected to assess the consistency of results. Medulloblastoma is a highly aggressive childhood tumor. Under constant experimental conditions, medulloblastoma cells were treated with 45 μM telmisartan and incubated for 48 hours (**figure 3d**). Based on PCR quantification results, a decrease tread in fold change was observed from 1 (control) to 0.02±0.02 (treated) (**figure 3d**). This decrease in the fold change can be attributed to the observed increase in the Ct values of *AGTR1* and *GAPDH* gene (Ct mean = 34.67±1.6, Ct mean=25.56±0.2), respectively, in treated cells and from *AGTR1* and *GAPDH* value (Ct mean=28.13±0.07, Ct mean= 24.60±0.07) respectively post-drug treatment in untreated cells (**figure 3d**). The rise in the Ct value indicates

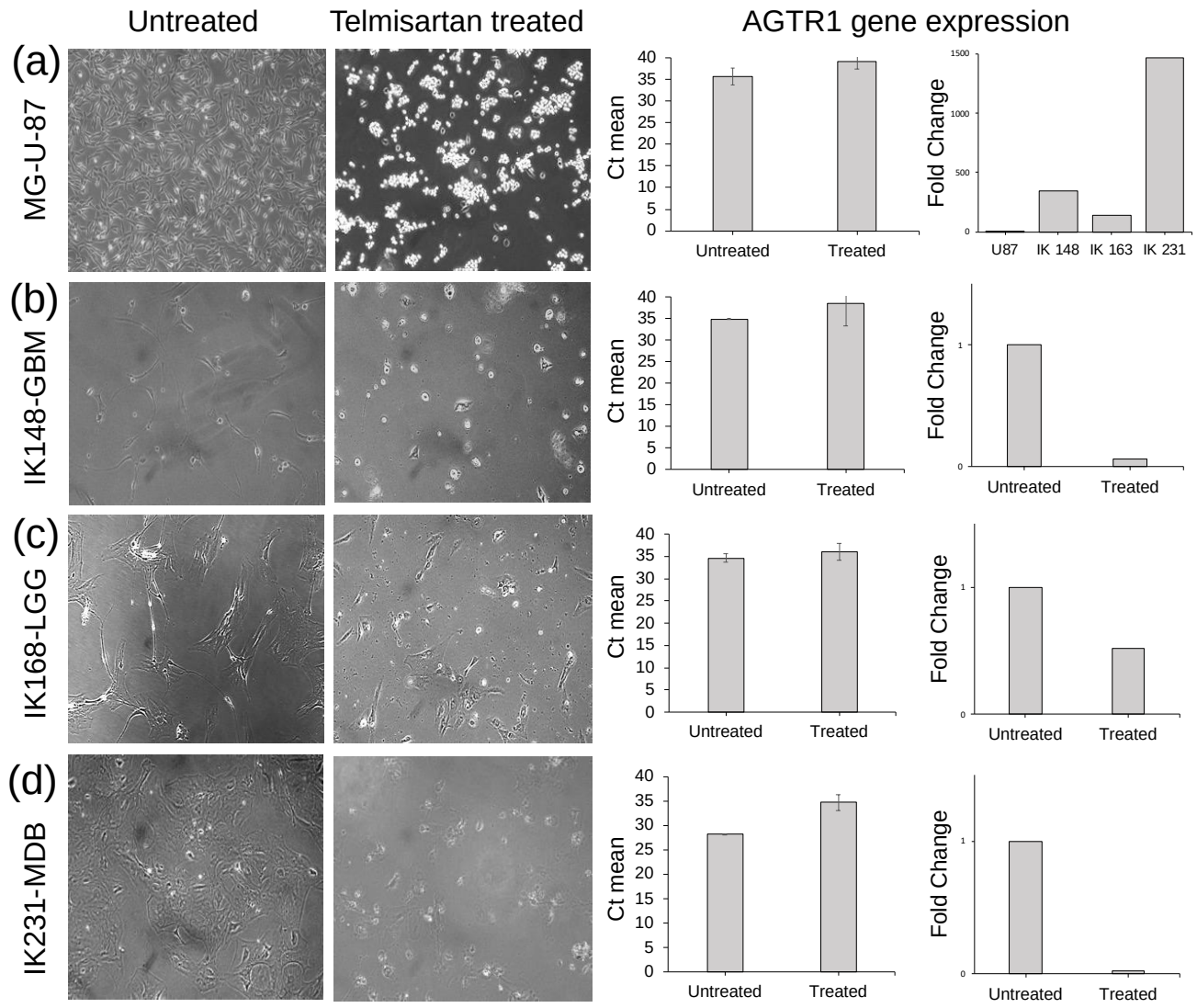

Figure 3: Invitro cell treatment with telmisartan at half maximal- inhibitory concentration to determine tumor cell response. (a) Represents untreated and treated cells of MG-U87 after 48hours incubation, followed by quantitative real time gene expression profile of AGTRI post 48 hours drug treatment in both treated and untreated cell where the mean Ct value is presented on vertical axis and treated and untreated groups on horizontal axis and the fold change observed in response to drug treatment. (b) Represents untreated and treated cells of IK148-GBM after 48hours incubation, followed by quantitative real time gene expression profile of AGTRI post 48 hours drug treatment in both treated and untreated cell and the fold change observed in response to drug treatment. (c) Represents untreated and treated cells of IK163-LGG after 48hours incubation, followed by quantitative real time gene expression profile of AGTRI post 48 hours drug treatment in both treated and untreated cell and the fold change observed in response to drug treatment. (d) Represents untreated and treated cells of IK231-MDB after 48hours incubation, followed by quantitative real time gene expression profile of AGTRI post 48 hours drug treatment in both treated and untreated cell and the fold change observed in response to drug treatment. In all three cases, the untreated cells showing clear morphology whereas after treatment with half-maximal inhibitory concentration, the cells are greatly affected in response to the presence of the drug.

that telmisartan did target the expression of the *AGTR1* gene in the glioma cell line.

4. Discussion

AGTR1, a G-protein coupled receptor, is essential for mediating the actions of Angiotensin II and maintaining various physiological activities in the human body. AGTR1 actively regulates blood pressure as a component of the renin-angiotensin system. It is reported that *AGTR1* is expressed in the brain, and it could influence the progression of brain cancer. The current study was designed after a thorough review of the existing literature confirming the presence of the renin-angiotensin system and the AGTR1 receptor in the brain, as well as its overexpression in gliomas. By specifically targeting the AGTR1 receptor in glioma cell lines, this study marks a novel attempt to repurpose telmisartan as an anticancer treatment.

The existence of AGTR1 in glioma has been confirmed by literature sources and several open databases. Furthermore, the findings of this study, using PCR analysis, confirmed the presence and overexpression of *AGTR1* genes compared to *GAPDH* in glioma tissue samples. *AGTR1* expression was found elevated in all three tested samples. According to histopathology studies acquired from the local hospital, these samples contained different types of gliomas, notably GBM, LGG, and medulloblastoma.

GBM has a well-documented history of aggressive behavior. It has been reported that elevated expression of *AGTR1 & 2* in GBM cell lines, which, when treated with angiotensin peptides, displays mitogenic response (Fogarty, Sánchez-Gómez, and Matute 2002). Although this gene is carcinogenic in several malignancies, its precise function in GBM is still unclear, prompting additional research (Singh et al. 2020). This study's results demonstrate this gene's high expression in both tissue and patient-derived primary GBM cell line. The second sample, a low-grade glioma, also showed elevated *AGTR1* expression in the present study, though at a lower level than the GBM sample. This finding is consistent with the notion that *AGTR1* overexpression is associated with gliomas' proliferative and infiltrative properties (Singh et al. 2020). As a result, the enhanced expression pattern of *AGTR1* in highly aggressive tumors, such as GBM, is likely to be more evident than in low-grade gliomas. Lastly, a malignant tumor originating in the cerebellum, medulloblastoma, primarily affects children. Despite the use of multimodal therapy techniques, many patients still encounter difficulties accessing the cure (Parsons et al. 2011). Given the aggressive character of this tumor, we expected an increase in *AGTR1* expression in our third sample, which was later confirmed to have considerable *AGTR1* overexpression.

Subsequently, to validate the hypothesis about the possible impact of inhibiting the overexpressed *AGTR1* gene in glioma cell line samples, a thorough analysis of the relevant literature was conducted to gather insights on the effects of AGTR1 inhibition in other tumor types. A study using rats found that inhibiting AGTR1 in glioma cells significantly reduced glioma development (Rivera et al. 2001). Hence existing angiotensin receptor blockers list was obtained and docked with AGTR1 structure from which telmisartan showed the highest binding affinity, which was selected to be repurposed as an anticancer drug. Various concentration (200μM, 100μM, 50μM, 25μM, 12.5μM, 6.25μM, and 0μM) of telmisartan was selected and administered in U-87 cell line. The results showed dose-dependent inhibition of AGTR1 in glioma cell lines; a decrease in the drug concentration increased the cancer cell survival rate. This is further supported by a study conducted by Masahide et al. (Matsuyama et al. 2010), where telmisartan (from 25-100 μM), applied for 72 hours, showed significant results

at a dose of 100μM. However, in this study, the concentration applied on the cell line was selected on the basis of IC_{50} value which was 14.82 ± 0.06 μM. Following the application of a 3(IC_{50}) μM dosage on the primary glioma cell line, it was found that cell proliferation decreased in all three samples, as evidenced by the corresponding changes in Ct values. These findings indicate that the drug is altering underlying pathways at a molecular level. Literature analysis of a study by Lucas et al. (Lee et al. 2014) looked at telmisartan's antiproliferative and apoptotic effects on human colon tumors. Telmisartan inhibited cell proliferation dose-dependently, resulting in lower cell survival across three human colon cell lines. Furthermore, Lucas et al. demonstrated that telmisartan therapy activated apoptosis in these cell lines by partially activating the peroxisome proliferator-activated receptor (PPAR γ). Takanori et al. (Matsui et al. 2019) demonstrated that telmisartan efficiently lowers the expression of *cyclin A2* and cyclin-dependent kinase 2 in human esophageal squamous cell carcinoma cell lines. This decrease in expression causes cell cycle arrest, specifically in the S phase, preventing the cell from progressing into the G2/M phase. More research is needed to understand the underlying mechanism through which telmisartan inhibits AGTR1 in brain tumors.

Further attempts were made to understand the fundamental causes of the differences in inhibition patterns in the selected samples. Because the cell lines used in this study represented many glioma subtypes, the observed variations in drug action can be attributable to this inherent diversity. The relationship between increased *AGTR1* expression and aggressive tumor features has been well established, with the angiotensin 2/AGTR1 complex thought to accelerate tumor growth (Paoletti, Bellone, and Zhou 2013). As a result, it was expected that the expression of the *AGTR1* gene would be increased in GBM and medulloblastoma, two of the most aggressive kinds of glioma. The present data revealed that *AGTR1* expression was substantially higher in both GBM and medulloblastoma than in LGG. Furthermore, medulloblastoma, known for its pediatric origin and high aggressiveness, showed elevated *AGTR1* expression. Given the increased expression levels in GBM and medulloblastoma, treating these tumors with AGTR1 antagonists should significantly influence cell proliferation, which is consistent with our findings. These findings demonstrate that telmisartan can be used to reduce the growth of glioma *in vitro* and can be used as a potential candidate for anticancer drugs.

5. Conclusions & Recommendation

We concluded from this study that telmisartan exhibits successful inhibitory effects against *AGTR1* expression in glioma cell lines. Given that this study was performed *in vitro* on patient-derived primary glioma cell lines, further research is imperative to validate the effects of telmisartan on cancer *in vivo* models. Furthermore, an in-depth investigation of underlying molecular pathways affected when AGTR1 is treated with its antagonist is warranted. Such an investigation would provide a comprehensive insight into glioma progression and use AGTR1 as a biomarker to design alternate cost-effective treatments.

Conflict of Interest

The authors declare that they have no competing interests.

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Study Approval

This study was approved by the ethical committee of Khyber Medical University, and an informed consent for each patient was received, dually signed by both the patient/guardian and researcher taking the consent.

Consent Forms

Consent forms are available with the authors.

Data Aavailability

All the data related to this study are available with the authors.

Authors Contribution

INK conceptualized the study, KK performed the experiments, SA and KU provided brain tumors samples, INK, JA, ZH, AJ, TK, DH, evaluated research plan, data analysis, reviewing and editing complete manuscript. INK, JA, ZH & AJ supervised the whole project.

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