Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.189*

Inhibition of the Angiotensin II Type 2 Receptor AT2R is a Novel Therapeutic Strategy for Glioblastoma

Richard Perryman[1#], Alexander Renziehausen[1#], Hamidreza Shaye[2,3#], Androniki D. Kostagianni[4], Antonis D. Tsiailanis[4], Tom Thorne[5], Maria V. Chatziathanasiadou[1,4], Gregory B. Sivolapenko[6], Mohamed Ahmed El Mubarak[6], Gye Won Han[3], Barbara Zarzycka[3,7], Vsevolod Katritch[3,8], Guillaume Lebon[9], Cristiana Lo Nigro[10], Laura Lattanzio[10], Sophie Morse[11], James Choi[11], Kevin O'Neill[1,12], Zoe Kanaki[13], Apostolos Klinakis[13], Tim Crook[1], Vadim Cherezov[2,3*], Andreas G. Tzakos[4,14*], and Nelofer Syed[1*]

[1]John Fulcher Neuro-Oncology Laboratory, Department Brain Sciences, Imperial College, London, UK; [2]Department of Chemistry, University of Southern California, Los Angeles, CA 90089, USA; [3]Bridge Institute, University of Southern California, Los Angeles, CA 90089, USA; [4]Department of Chemistry, University of Ioannina, Ioannina, Greece; [5]Department of Computer Science, University of Surrey, Surrey, UK; [6]Laboratory of Pharmacokinetics, Department of Pharmacy, University of Patras, Patras, Greece; [7]Division of Medicinal Chemistry, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherland; [8]Department of Quantitative and Computational Biology, University of Southern California, Los Angeles, CA 90089, USA; [9]Insitut de Génomique Fonctionnelle, University of Montpelier, CNRS, INSERM, Montpellier, France; [10]Department of Oncology, Ospedale San Croce e Carle, Cuneo, Italy; [11]Department of Bioengineering, Imperial College London, London, UK; [12]Department of Neurosurgery, Charing Cross Hospital, London, UK; [13]Centre for Basic Research, Biomedical Research Foundation of the Academy of Athens, Greece; [14]University Research Center of Ioannina (URCI), Institute of Materials Science and Computing, Ioannina, Greece

Introduction

The prognosis for patients with glioblastoma (GBM) remains poor, therefore novel therapeutic approaches to address this unmet clinical need are urgently required. We illustrate that angiotensin II (AngII), a peptide involved in salt and water balance, is produced endogenously by GBM cells and promotes proliferation of these cells, via the type 2 receptor of AngII (AT_2R). We repurposed EMA401, a peripherally restricted AT2R selective antagonist, originally developed for the treatment of neuropathic pain. We show that EMA401 efficiently inhibits proliferation of GBM spheroids that express AT2R, and prevents both invasiveness and angiogenesis. We enhance the central nervous system (CNS) penetration of EMA401, through the generation of a novel compound, A3E. The novel compound demonstrated superior efficacy both *in vitro* and *in vivo* with minimal toxicity. This study demonstrates that inhibition of AT_2R is a new and promising candidate target for systemic therapy of GBM [1].

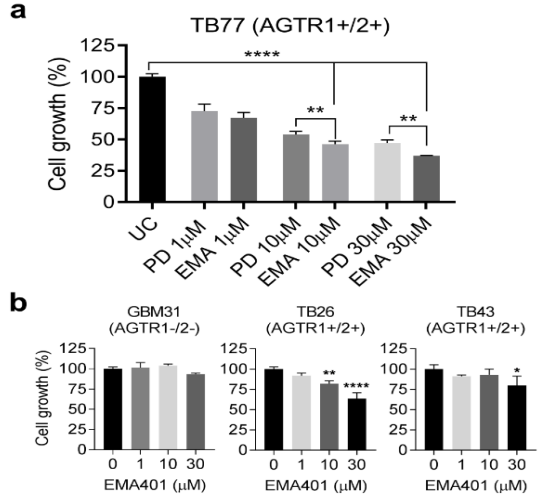

Fig. 1. EMA401 significantly inhibits the growth of GBM cells via AT2R.

Results and Discussion

Here we show that AT_2R signalling [2], has an significant role in promotion of

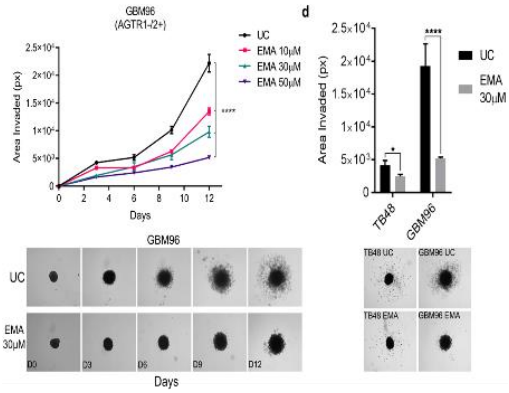

Fig. 2. Evaluating spheroid growth, spheroid invasion and the ability of GBM cells to induce angiogenesis when treated with angiotensin receptor antagonists.

GBM tumor growth and invasiveness. This study follows our previous work in which we reported that AT_2R inhibition with EMA401 inhibits proliferation, invasiveness, and angiogenesis in metastatic melanoma [3]. The AngII receptors differ functionally between cancer types. Furthermore, the effects of each receptor also depend on the microenvironmental stressors present in the vicinity of cancer cells. Here we show that under conditions of starvation stress (the norm in most tumor microenvironments), AT_2R adopts a promoting function, therefore selective antagonism has anti-tumor effects.

Herein, we reported that in low serum conditions, AngII minimally increased the growth of GBM lines and primary cultures which expressed AT1R but not AT2R. Selective antagonism of AT2R by PD123319 blocked the enhanced growth conferred by exogenous AngII, but no effects were observed with treatment with losartan, which is a selective AT1R antagonist. Our results indicate that under growth factor limiting conditions, AngII confers a growth advantage to GBM which is transduced via AT2R. PD123319 inhibited the proliferation of AT2R expressing cells in the absence of exogenous AngII, and effects were significant in two of these cell lines (8MG: $P<0.05$ and TB77: $P<0.0001$). These results demonstrate that under low serum conditions, autocrine production of AngII promotes cell growth of GBM cells via AT2R signaling.

EMA401 demonstrated significantly more potent growth inhibition than PD123319 ($P=0.0011$). Similar activity was observed in additional primary GBM cultures expressing AGTR2 (TB26, TB43). Notably, the AGTR2 negative GBM31 cell line was insensitive to EMA401. EMA401 significantly reduced the invasiveness of GBM96 cells in a dose-dependent manner. TB48 spheroids exhibited

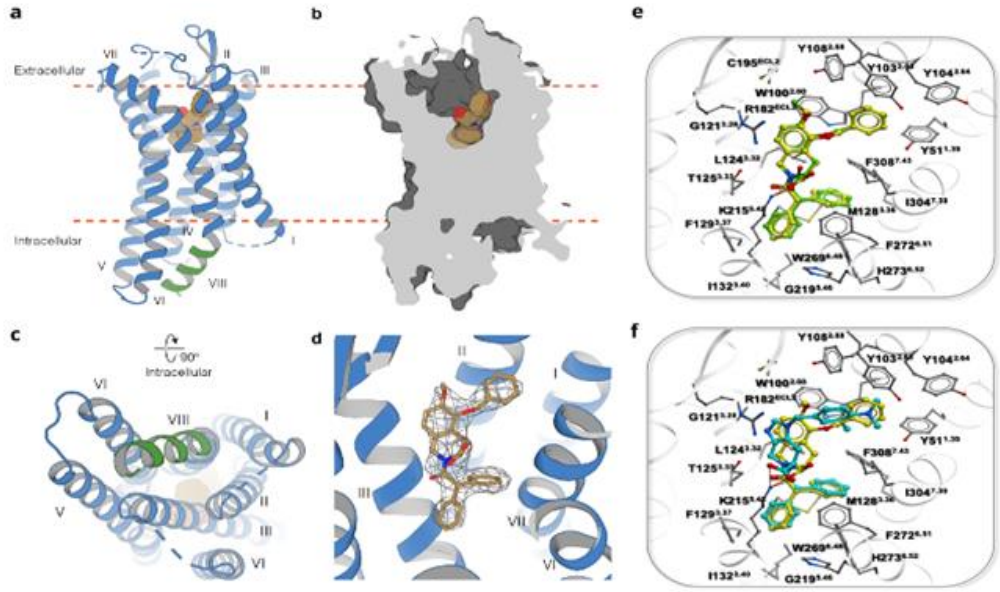

Fig. 3. AT2R-EMA401 structure and details of AT2R-ligand interactions.

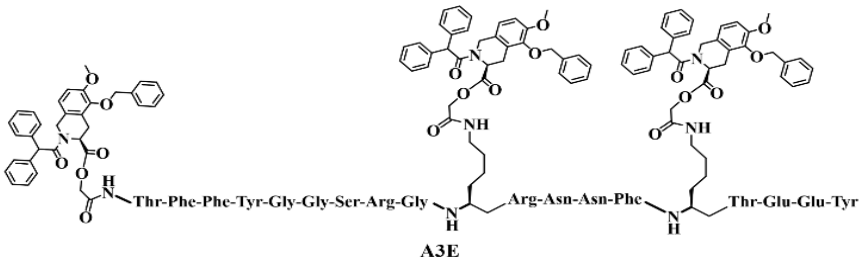

Fig. 4. Structure of A3E (Angiopep-2-EMA401).

lower invasive properties than GBM96, but invasiveness was nonetheless significantly reduced by 30 μM EMA401 (Figures 1,2).

Next, we determined the crystal structure of EMA401 bound to AT2R. The AT2R structure is comprised of an heptahelical transmembrane bundle (7TM helices I-VII), three extracellular loops (ECLs 1-3), three intracellular loops (ICLs 1-3), and an intracellular amphipathic helix VIII. The crystal structure of AT_2R bound to EMA401 was determined and revealed the receptor to be in an active-like conformation with helix-VIII blocking G protein or β-arrestin recruitment. Based on our X-ray structure of EMA401 bound to AT2R (Figure 3), it is evident that its carboxylic acid group is essential for forming interactions with key AT2R residues.

Despite the inhibitory effect of EMA401 in GBM promotion, a potential obstacle to its clinical use of

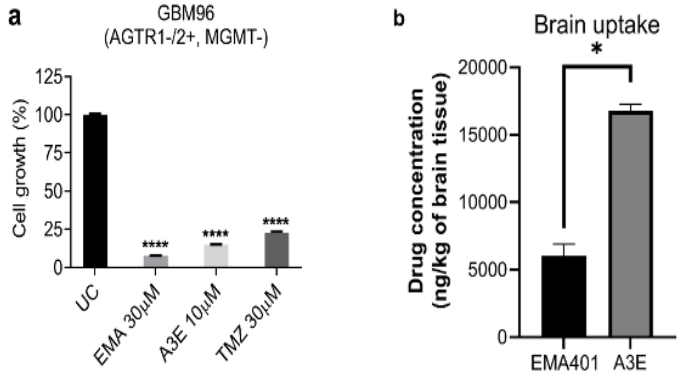

Fig. 5. a) The effects of EMA401 and A3E on proliferation in primary GBM cultures b) Brain biodistribution of EMA401 after intravenous injection of EMA401, and A3E.

is the reported inability of the molecule to efficiently penetrate the intact BBB [4]. To enhance the likelihood of using EMA401 as a potential GBM therapeutic, either a structural derivatization or proper formulation has to be performed. On the basis of our X-ray structure of EMA401 bound to AT2R, it is evident that its carboxylic acid group is essential for forming interactions with key AT2R residues. We therefore use this group as a reactive handle to conjugate three EMA401 units, via cleavable ester bonds to Angiopep-2, to generate A3E. (Figure 4) Angiopep-2 is a molecule that potentiates the CNS delivery of drugs typically unable to pass the BBB [5-7].

The *in vitro* anti-proliferative effect of A3E was at least as potent as EMA401 in all primary GBM cell lines that has been tested. In addition, through biodistribution studies we demonstrated that A3E notably increased CNS penetration of EMA401 with respect to the native EMA401 (Figure 5).

Together, these observations prompted us to test A3E
in an orthotopic model of GBM. A3E demonstrated a clear inhibitory effect on tumor growth, whereas native EMA401 did not affect tumor size, due to its inability to penetrate the BBB. Moreover, weight (a marker of systemic impact of tumor growth) was preserved in mice treated with A3E but not in controls or EMA401 treated mice (Figures 6 and 7)**.**

Overall, this study validates AT2R as a viable therapeutic target in GBM and affirm A3E as a leading candidate for further clinical development. Given the high frequency of AT2R expression, our results support further investigation of the RAS and its therapeutic modulation in GBM.

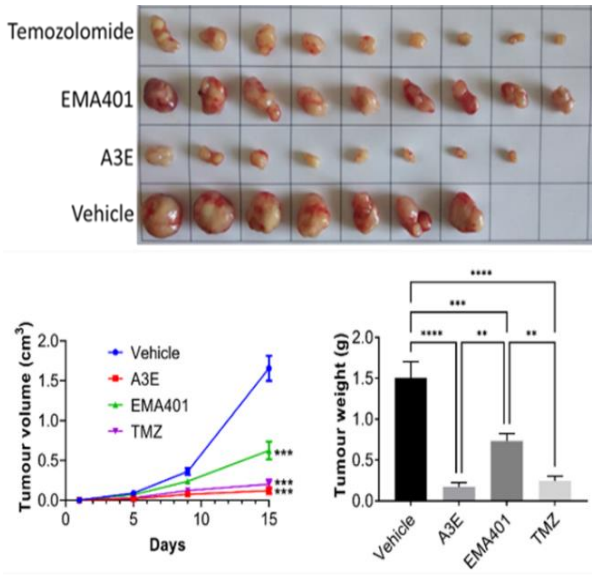

Fig. 6. Average volume and weight of subcutaneous U87-GFP/luc tumors in NSG mice after 21 days of treatment with EMA401, A3E, TMZ (30 mg/kg, n = 9) or vehicle.

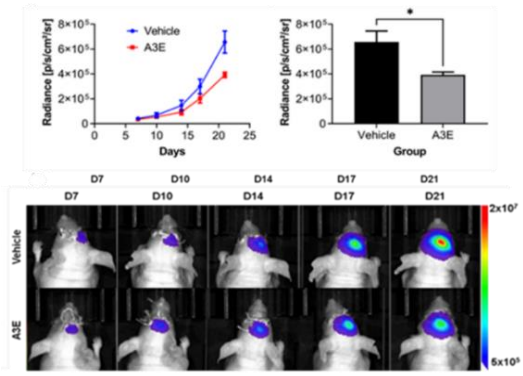

Fig. 7. Immunohistochemical staining of sections from mice treated with A3E.

Acknowledgments

The project/research is co-financed by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: 991, acronym PROTECT to AGT). The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grants (Fellowship Numbers: 14 and 1075).

References

1. Perryman, R., et al. *Proceedings of the National Academy of Sciences* **119**(32), e2116289119 (2022), https://doi.org/10.1073/pnas.2116289119
2. Valenzuela, R., et al. *Cell Death & Disease* **7**(10), e2427-e2427 (2016), https://doi.org/10.1038/cddis.2016.327
3. Renziehausen, A., et al. *Oncogene* **38**(13), 2320-2336 (2019), https://doi.org/10.1038/s41388-018-0563-y
4. Anand, U., et al. *Eur J Pain* **17**(7), 1012-1026 (2013), https://doi.org/10.1002/j.1532-2149.2012.00269.x
5. Wang, L., et al. *J Drug Target* **23**(9), 832-846 (2015), https://doi.org/10.3109/1061186X.2015.1025077
6. Figueiredo, P. et al. *Int J Pharm* **511**(2), 794-803 (2016), https://doi.org/10.1016/j.ijpharm.2016.07.066
7. Gao, H., et al. *Mol Pharm* **11**(8), 2755-2763 (2014), https://doi.org/10.1021/mp500113p