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FULL PAPER

The effect of exposure to electric fields on EGFR and PDGFR in glioblastoma cell model: In vitro study

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The aim of this study was to determine differences in expression of epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR)) in glioblastoma cell cultures *in vitro* when exposed to electric fields. This work followed a true experimental research design with a post-test only control group design. Glioblastoma cell culture was divided into 2 groups, namely the glioblastoma cell culture group exposed to electric field and those that were not exposed. This tool provided a voltage of 10, 30, and 50 peak to peak Voltage (V_{pp}) with a frequency of 100 KHz alternating current (AC). Evaluation of EGFR and PDGFR expressions was carried out after exposure. Exposure to a weak electric field with medium frequency for 24, 48, and 72 hours decreased the expression of EGFR and PDGFR in the angiogenesis process of glioblastoma cell cultures *in vitro* but not significantly. Further research is needed with a larger range of doses and treatment duration.

KEYWORDS

Glioblastoma; EGFR; PDGFR; in vitro; electric fields.

Introduction

Electric field therapy is one of the latest therapeutic methods for treating cancer [1]. Non-contact Electro-Capacitive Cancer Treatment (ECCT) and Tumor-Treating Field (TTF) have been introduced and researched for cancer treatment [2,3]. Non-contact exposure to low-voltage (1-3 V/cm) and intermediate-frequency (100-300 kHz) alternating electric fields in the tumor area can inhibit tumor progression [4]. TTF itself has been commonly used as approved by the Food and Drug Administration (FDA) with results without side effects [5]. Glioblastoma is a malignant tumor of the central nervous system originating from glial cells with high mitotic activity accompanied by microvascular proliferation [6,7].

Glioblastoma has a very heterogeneous histological picture so it is also called Glioblastoma Multiforme (GBM) [8,9]. Cases of central nervous system (CNS) tumors were reported at 23.03 cases per 100,000 population. As many as 1.4% of the total number of cancer incidents in the United States are primary brain tumors. Every year, there are 20,500 new diagnoses and 12,500 deaths due to brain tumors in the United States. GBM occurs in 3.19 per 100,000 residents in the United States, and is the most common malignant primary brain tumor [10]. Retrospective research about brain tumor profile at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia in 2012-2018 showed that 1540 patients were diagnosed with primary brain tumors. GBM itself accounts for around 5.3% of all tumors and 41.8% of all gliomas [11].

Angiogenesis is the formation of new blood vessels by creating new routes or remodeling existing blood vessels [12,13]. Angiogenesis in glioblastoma will determine the growth rate and progressive nature of the tumor cells [14,15]. Angiogenesis is currently an important therapeutic target for glioblastoma [16,17]. One of the pathways most frequently involved is the receptor tyrosine kinases (RTK) pathway [18,19]. RTK will bind epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) [19-21]. EGFR and PDGFR are the two receptors with the most mutations expressed [22-23]. Decreased RTK activity will reduce EGFR and PDGFR. This decrease in EGFR and PDGFR levels is expected to reduce the angiogenesis process in GBM cells [24]. This study aims to prove differences in the expression of EGFR and PDGFR in glioblastoma model cell cultures *in vitro* when exposed to an electric field.

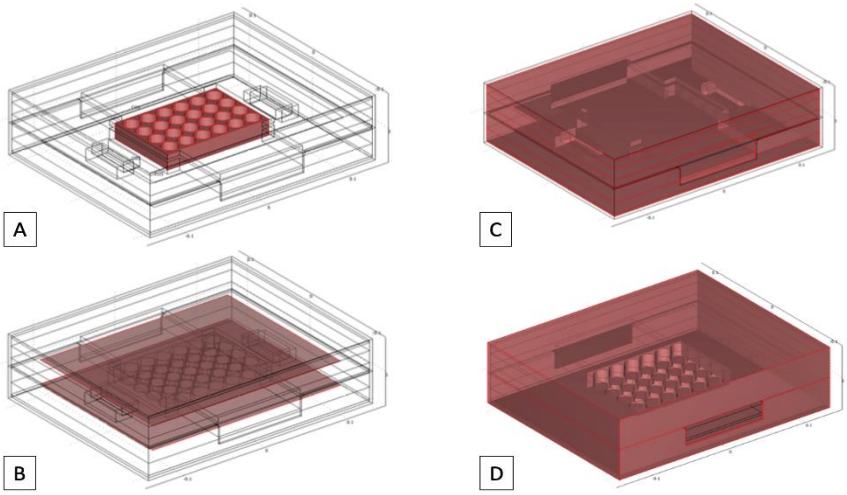

FIGURE 1 The illustration of electric field box. The component consists of 24-well microplate (A), electrodes (B), ethylene vinyl acetate foam (C), and acrylic outer layer (D). The device consisted of microplate components, ethylene vinyl acetate foam, electrodes and acrylic, and had a conductor that transmited and received electric current. This device was used to generate a static electric field with non-contact electrodes

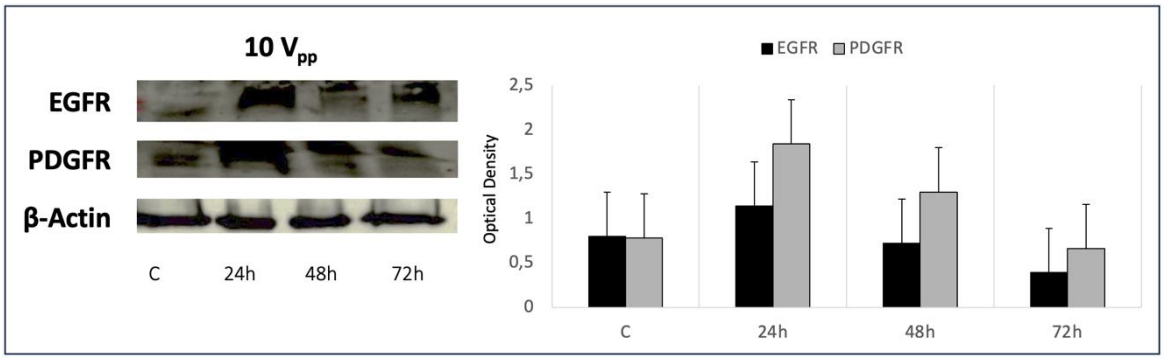

FIGURE 2 Western blot and optical density of expression of EGFR and PDGFR in U87 cell line treated with 10 V_{pp} electric field

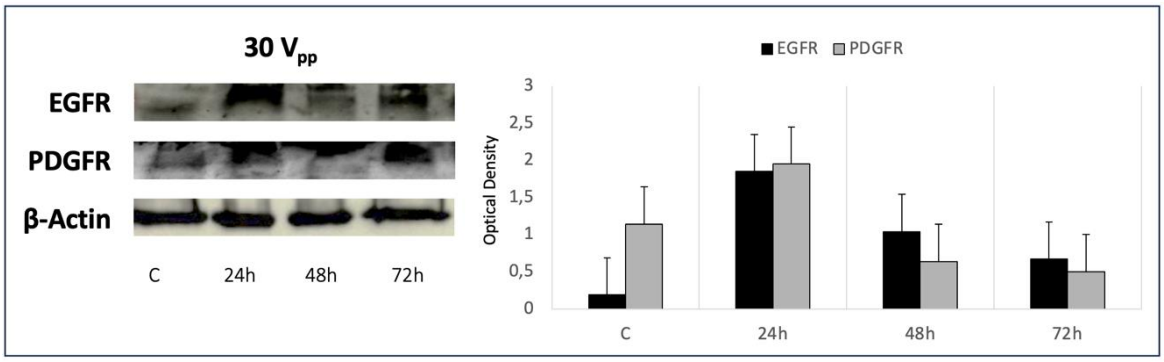

FIGURE 3 Western blot and optical density of expression of EGFR and PDGFR in U87 cell line treated with 30 V_{pp} electric field

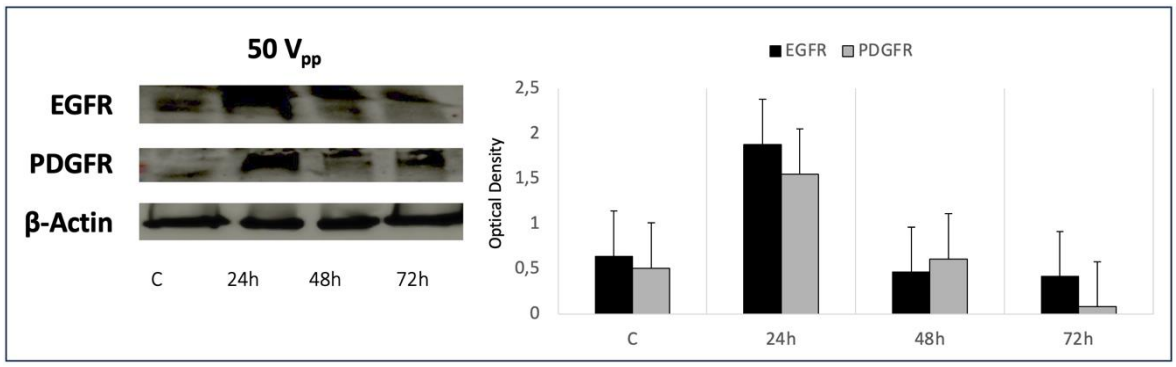

FIGURE 4 Western blot and optical density of expression of EGFR and PDGFR in U87 cell line treated with 50 V_{pp} electric field

Experimental

Study design and objective

This research is true experimental research. There were twelve experimental groups in this study (eight treatment groups and four control groups). The number of samples used for each group was 12. The research aimed to determine the effect of exposure to a weak electric field with medium frequency on the angiogenesis of glioblastoma cell cultures *in vitro*.

Hypothesis and originality

The hypothesis of this study predicts that electrical fields therapy reduces EGFR and

PDGFR expression in GBM *in vitro*. Currently there are no previous studies that discuss this particular topic.

Setting of the electric fields

Modifications to the culture plate were made into 24 wells to accommodate 2 replications of 12 samples for each group. This tool provided 10, 30, and 50 peak-to-peak voltage (V_{pp}) (low intensity) with a frequency of 100 KHz (intermediate frequency) alternating current (AC). The type of electric field provided is a static electric field. A pair of capacitive electrodes positioned flanking on top and bottom of the microplate was connected to a square function oscillator. One-directional field was set up to 10 V_{pp}, 30 V_{pp}, and 50 V_{pp} and they generated between the pair of capacitive electrodes. The electrode plate did not touch the culture cells but was placed on treatment container. This allowed the cell not to be electrified by the electric current of the electrode. Cell cultures were treated with external electrostatic wave and incubated for 24, 48, and 72 hours. A control group with 4 replications was also incubated at the same time.

The components of the device are illustrated in Figure 1 (made by CTECH Laboratories, Tangerang, Indonesia). The electric field intensity and frequency in the medium were calculated using an oscilloscope. The device has been calibrated with the desired intensity and frequency in Sepuluh Nopember Institute of Technology (ITS), Surabaya, Indonesia.

Cell line preparation

A cell line is a cell culture that has been conditioned to continue to proliferate. This research used tumor-derived cell lines. Tumor-derived cell lines have been widely used for research and are authentic to the tumor of origin. The glioblastoma cell line type U87MG is a cell line developed from grade III astrocytoma (distributed by American Type Culture Collection (ATCC)). U-87 MG is a cell line with epithelial morphology which originates from a male patient. The U87MG cell line is one of the most widely used cellular models in research because its genomic characteristics and phenotype resemble human glioblastoma cells. This cell line was grown in Dulbecco modified eagle medium (DMEM) with 2 mM glutamine, 1.5 g/l sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, and fetal bovine serum (FBS) 10% (Life Technologies, Prague, Czech Republic). U87-MG was stored at room temperature under stable conditions at 37 ^{o}C, 95% humidity, and 5% CO_2 with treatment of the medium being changed two to three times per week.

The cell line was implanted in a Cryotube at -80 ^{o}C which had been acclimatized at the Stem Cell Laboratory, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia. The cell suspension was moved into the wells. One microliter suspension was placed in 24-well microplate loaded with 999 µL medium with total 5 x 105 cells/ mL. Cell cultures were treated with noncontact electric fields with 10, 30, and 50 V_{pp} and incubated for 24, 48, and 72 hours with 12 replications each.

EGFR and PDGFR antibody and Western blot

Western blot procedure is used to identify proteins and mark target proteins using antibodies. Western blot was carried out at the Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. Primary rabbit polyclonal antibodies to EGFR (SAB4500711, Sigma Aldrich, Missouri, United States) and PDGFR (HPA028499, Sigma Aldrich, Missouri, United States) were used.

Statistical analysis

This research is a study with a post-test only control group design. Western blot qualitative

images were converted into quantitative data using the ImageJ application (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation). EGFR and PDGFR data were presented in the form of relative expression graphs. Kolmogorov-Smirnof analysis was carried out for data normality and ANOVA for analysis of collected data. The author uses SPSS 26 software (IBM Corporation) for data processing. Significance of results were followed by a post-hoc test. This study has received ethical approval from the Research Ethics Dr. Soetomo General Academic Hospital No. 1522/KEPK/IX/2019.

Results and discussion

EGFR and PDGFR expression in U87MG glioblastoma cell lines

We measured the expression of EGFR and PDGFR on each voltage and hour of treatment using western blot. ImageJ converted western blot bands into optical density numbers. The results of the western blot examination of EGFR and PDGFR can be seen in Figures 2-4. Cells exposed to a dose of 10 V_{pp} experienced a decrease in the average expression of both EGFR and PDGFR, as the exposure time increased.

Correlation analysis between EGFR and PDGFR in U87MG glioblastoma cell lines

We performed correlation analysis between EGFR and PDGFR expression for voltage and treatment hour time. As presented in Tables 1-3, there were no significant correlations between increasing voltage and hours of treatment on EGFR and PDGFR expression although the trend was decreasing.

Electrical field treatment, tumor treatment and angiogenesis

Electric field therapy has been previously studied for its benefits in cancer treatment, such as Electro-Capacitive Cancer Treatment (ECCT) and Tumor-Treating Field (TTF) [2,3]. The latter itself has been approved by the Food and Drug Administration (FDA) in the United States. The field dose generator produces electricity with medium frequency (100-300 kHz) and very low intensity of < 2 V/cm. Kirson *et al.* reported that the therapeutic dose for glioma cells was 200 kHz [4]. Electricity with a frequency range of 100 kHz to 1 MHz has a significant effect on dividing cells [25]. The use of medium frequency electricity with very low intensity aims to avoid stimulation of cells that are easily excited and prevent an increase in tissue temperature. Increasing the frequency by > 1 kHz above the acceptable range shortens the time between depolarization and hyperpolarization [26]. Increasing the frequency to the MHz range will cause a loss of cell dielectric properties and cause an increase in tissue temperature [27].

TTF prolongs survival when used in combination with TMZ (Temozolamide) [28,29]. Electric field therapy also disrupt angiogenesis, a key process for tumor growth and progression, via receptor tyrosine kinases (RTKs) pathways, thus identifying potential synergism with anti-angiogenic agents (e.g., bevacizumab, cediranib, enzastaurin, and sunitinib) [30, 31]. RTKs are the most common oncogenic pathways in glioblastoma, which then bind to growth factors (GF) [32].

Two gene factors that are frequently mutated in RTK pathways are EGFR and PDGFR [32]. The EGFR is responsible for maintaining cell survival, and is important for differentiation, proliferation and migration of central nerve cells [33]. Overexpression of receptors or ligands, receptor mutations, and/or amplification of the EGFR locus can activate EGFR signalling in GBM cells [34]. Specifically in humans, PDGFR-α is in glial tumor cells while PDGFR-β is in the vasculature [35]. A study conducted by Popescu et al showed an important correlation between PDGFR and neo-

angiogenesis in glioblastoma [36]. Research carried out on GBM cells *in vitro* shows that the results of the analysis show that PDGFR inhibition results in GBM cell death.

EGFR expression

EGFR expression levels in this study generally decreased with increasing exposure time at doses of 10, 30, and 50 V_{pp}, but this was not statistically significant. Currently there are no other studies that discuss changes in EGFR expression after exposure to electric fields with TTF, ECCT or other electric field therapies. Kim *et al.* conducted research on one of the angiogenesis factors, VEGF, in the GBM cell lines U-373 and U-87. The two cell lines were exposed to a TTF electric field with an intensity of 1.75 V/cm and a frequency of 200 kHz for 24, 48 and 72 hours, and angiogenesis was evaluated using western blot, zymography and qRT-PCR. Kim *et al.* found that TTF attenuated the expression of HIF1α and VEGF in cells treated with TTF. It can be concluded that TTF can influence the angiogenesis process of GBM cells [37].

Another study also addressed the effect of TTF on EGFR expression in lung adenocarcinoma tumors. Giladi et al studied non-small cell lung cancer (NSCLC) samples in vitro at a dose of 150 kHz for 72 hours [4]. Compared with Erlotinib exposure alone, the combination of TTF and Erlotinib caused a significant reduction in the viability of HCC827 (mutant EGFR) cells. Other research also metioned that administration of TTF (1.5 V/cm, 150 kHz, 48 hours) affects the angiogenesis process in osteosarcoma cells *in vitro*. This study concluded that TTF has the ability to suppress VEGF and MMP2 in 2H11 and osteosarcoma cell lines through the angiogenesis process [38].

One of the underlying hypothesis why in this study electric field therapy was able to reduce EGFR but not significantly is that angiogenesis is not the main mechanism of action of TTF or other electrical therapy and tumorigenesis of GBM [39]. The mechanisms of action of TTF and similar electrical therapies mainly include anti-mitosis, DNA repair interruption, autophagy, immunogenicity, anti-migration, and cell membrane permeability interruption [40]. *In vitro* and *in vivo* experiments have shown that alternating electric fields in the frequency range of 100-300 kHz have the main specific effect of prolonging mitosis, which leads to the cessation of cell proliferation and can cause rupture of cell membranes [41].

PDGFR expression

In this study, PDGFR levels also decreased with increasing exposure time, but this was not statistically significant. Currently, there are no other studies that discuss changes in PDGFR expression after exposure to electric field therapy. Kim *et al.* demonstrated that TTF can inhibit the invasion, migration, and angiogenesis of GBM cells through downregulating phosphoinositide 3-kinase (PI3K) signalling of GBM cells [37]. The mitogen-activated protein kinase (MAPK) and PI3K pathways control proliferation, invasion and migration and tumorigenesis [42].

Over-expression of PDGFR promotes angiogenesis of endothelial progenitor cells through PI3K signaling pathway [43]. In animal model studies, increased expression of PDGFR was seen in mice modified in the glial fibrillary acidic protein (GFAP) promoter gene. This increase in growth factors is experienced by astrocyte cells but has been shown to provide different results regarding oncogenic effects. Specifically in humans, PDGFR-α is in glial tumor cells while PDGFR-β is in the vasculature. Further research shows that PDGFR can independently cause malignant growth of glial cells. It has been proven that transgenic mice with over-expression of PDGFR cause brain tumors [44]. Therefore, it is logical that if TTF can interfere with the PI3K pathway, PDGFR expression will also be disrupted.

Kim *et al.* observed a time-dependent decrease in PI3K and MAPK signalling, with a concomitant decrease in cell proliferation and migration. The study is the first report describing the potential biological mechanism of TTF in inhibiting GBM cell invasion and migration [37]. This is supported by the results of this study which decreased with the lengthening of post-exposure time to electric field therapy. Another kinomics study also suggested that electric field therapy combined with the PDGFR inhibitor, crenolanib, reduced PDGFR-α activity when compared to either treatment alone [45]. This also provides opportunities for further research regarding combination electrical field therapy with chemotherapy agents.

TABLE 1 Post-Hoc analysis of expression of EGFR and PDGFR in 10 V_{pp} groups

Antibody and Voltage	Group	Mean±SD	Comparison Group	Mean±SD	*P*-value
EGFR 10 V_{pp}	Control	0.80±0.62	24-hour	1.14±0.43	1
			48-hour	0.72±0.19	1
			72-hour	0.39±0.36	1
PDGFR 10 V_{pp}	Control	0.788±0.13	24-hour	1.84±0.27	0.4
			48-hour	1.30±0.34	1
			72-hour	0.66±0.05	1

TABLE 2 Post-Hoc analysis of expression of EGFR and PDGFR in 30 V_{pp} groups

Antibody and Voltage	Group	Mean±SD	Comparison Group	Mean±SD	*P*-value
EGFR 30 V_{pp}	Control	0.19±0.15	24-hour	1.84±0.97	0.046
			48-hour	1.04±0.81	1
			72-hour	0.67±0.69	1
PDGFR 30 V_{pp}	Control	1.14±0.43	24-hour	1.95±1.50	0.4
			48-hour	0.64±0.31	1
			72-hour	0.50±0.38	1

TABLE 3 Post-Hoc analysis of expression of EGFR and PDGFR in 50 V_{pp} groups

Antibody and Voltage	Group	Mean±SD	Comparison Group	Mean±SD	*P*-value
EGFR 50 V_{pp}	Control	0.64±0.55	24-hour	1.88±1.05	0.603
			48-hour	0.46±0.38	1
			72-hour	0.41±0.67	1
PDGFR 50 V_{pp}	Control	0.51±0.27	24-hour	1.55±0.30	0.49
			48-hour	0.61±0.41	1
			72-hour	0.08±0.08	1

Conclusion

Electric field therapy may have potential as GBM cell therapy by targeting the angiogenesis process. This is supported by the results of this study which show a decreasing trend in the expression of EGFR and PDGFR after exposure to electric fields. Other studies have also discussed the beneficial effects of electric fields therapy on angiogenesis [25-45]. Currently, there are no previous studies that discuss EGFR and PDGFR expression after exposure to electric field therapy in GBM. Therefore, the results of this study have no comparison discussion to compare with previous studies. The authors suggest further research in the future with a wider range of doses and duration of exposure. Similar research can also be carried out further *in vivo* or experimental animals.

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Authors' Contributions

Conceptualization, data analysis, and drafting: Asra Al Fauzi, Khrisna Rangga Permana, and Irwan Barlian Immadoel Haq. Review and revising: Muhammad Arifin Parenrengi, Purwati, Budi Utomo, and Sahudi. Electric box device: Warsito Purwo Taruno.

Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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References

[1] S. Zhong, S. Yao, Q. Zhao, W. Zhuo, Z. Liu, L. Li, Electricity-assisted cancer therapy: From traditional clinic applications to emerging methods integrated with nanotechnologies, *Advanced NanoBiomed Research,* **2023**, *3*, 3. [Crossref], [Google Scholar], [Publisher]

[2] O.R. Hasbullah, I. Fiddiyanti, D.R. Handayani, E. Sutedja, D. Ismono, N.N. Hidajat, W. Widowati, E. Afifah, H.S.W. Kusuma, R. Rizal, F. Alamsyah, W.P. Taruno, Non-contact electric field exposure provides potential cancer therapy through p53-independent proliferation arrest and intrinsic pathway apoptosis induction in MG-63 cell lines, *Hayati*, **2023**, *30*, 3, 522–531. [Crossref], [Google Scholar], [Publisher]

[3] S. Shams, C.B. Patel, Anti-cancer mechanisms of action of therapeutic alternating electric fields (tumor treating fields [TTFields]), *Journal of Molecular Cell Biology,* **2022**, *14,* 8. [Crossref], [Google Scholar], [Publisher]

[4] M. Giladi, U. Weinberg, R.S. Schneiderman, Y. Porat, M. Munster, T. Voloshin, R. Blatt, S. Cahal, A. Itzhaki, A. Onn, E.D. Kirson, Y. Palti, Alternating electric fields (tumor-treating fields therapy) can improve chemotherapy treatment efficacy in non-small cell lung cancer both in vitro and in vivo, *Seminars in Oncology,* **2014**, *41*, 35–41. [Crossref], [Google Scholar], [Publisher]

[5] Y. Jo, S.-G. Hwang, Y.B. Jin, J. Sung, Y.K. jeong, J.H. Baek, J.M. Cho, E.H. Kim, M. Yoon, Selective toxicity of tumor treating fields to melanoma: an in vitro and in vivo study, *Cell Death Discovery,* **2018**, *4*, 46. [Crossref], [Google Scholar], [Publisher]

[6] R. Tamma, G. Ingravallo, T. Annese, A. Damati, L. Lorusso, D. Ribatti, Tumor microenvironment and microvascular density in human glioblastoma, *Cells,* **2022**, *12*, 11. [Crossref], [Google Scholar], [Publisher]

[7] A. Zottel, I. Jovčevska, N. Šamec, R. Komel, Cytoskeletal proteins as glioblastoma biomarkers and targets for therapy: A systematic review, *Critical Reviews in Oncology/Hematology*, **2021**, *160*, 103283. [Crossref], [Google Scholar], [Publisher]

[8] V.E. Mikkelsen, O. Solheim, Ø. Salvesen, S.H. Torp, The histological representativeness of glioblastoma tissue samples, *Acta*

Neurochirurgica (Wien) **2021**, *163*, 1911–1920. [Crossref], [Google Scholar], [Publisher]
[9] A. Becker, B. Sells, S. Haque, A. Chakravarti, Tumor heterogeneity in glioblastomas: From light microscopy to molecular pathology, *Cancers (Basel)* **2021**, *13*, 761. [Crossref], [Google Scholar], [Publisher]
[10] Q.T. Ostrom, G. Cioffi, K. Waite, C. Kruchko, J.S. Barnholtz-Sloan, CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2014–2018, *Neuro Oncology* **2021**, *23*, 1-105. [Crossref], [Google Scholar], [Publisher]
[11] J. Wahyuhadi, M.F. Raiyan Pratama, R.T. Zahra Wathoni, H. Basuki, The Indonesian central nervous system tumors registry (Ina-CTR) : 7 years result from single institution of primary brain tumor epidemiology, *Indonesian Journal of Neurosurgery* **2021**, *4*, 1. [Crossref], [Google Scholar], [Publisher]
[12] M.C. Tate, M.K. Aghi, Biology of angiogenesis and invasion in glioma, *Neurotherapeutics,* **2009**, *6*, 447–457. [Crossref], [Google Scholar], [Publisher]
[13] C. Benazzi, A. Al-Dissi, C.H. Chau, W.D. Figg, G. Sarli, J.T. Oliveira, F. Gartnerr, Angiogenesis in spontaneous tumors and implications for comparative tumor biology, *The Scientific World Journal,* **2014**, *2014*, 1–16. [Crossref], [Google Scholar], [Publisher]
[14] T. Würdinger, B.A. Tannous, Glioma angiogenesis, *Cell Adhesion & Migration* **2009**, *3*, 230–235. [Crossref], [Google Scholar], [Publisher]
[15] A. Mosteiro, L. Pedrosa, A. Ferrés, D. Diao, A. Sierra, J.J. Gonzalez, The vascular microenvironment in glioblastoma: A comprehensive review, *Biomedicines*, **2022**, *10*, 1285. [Crossref], [Google Scholar], [Publisher]
[16] A.S. Chi, A.G. Sorensen, R.K. Jain, T.T. Batchelor, Angiogenesis as a therapeutic target in malignant gliomas, *The Oncologist,* **2009**, *14*, 621–636. [Crossref], [Google Scholar], [Publisher]
[17] E. Balandeh, K. Mohammadshafie, Y. Mahmoudi, M.H. Pourhanifeh, A. Rajabi, Z. Razaghi Bahabadi, A.H. Mohammadi, N. Rahimian, M.R. Hamblin, H. Mirzaei, Roles of Non-coding RNAs and Angiogenesis in Glioblastoma, *Frontiers in Cell and Developmental Biology,* **2021**, *9*, 1. [Crossref], [Google Scholar], [Publisher]
[18] M. Tilak, J. Holborn, L.A. New, J. Lalonde, N. Jones, Receptor tyrosine kinase signaling and targeting in glioblastoma multiforme, *International Journal of Molecular Sciences,* **2021**, *22*, 1831. [Crossref], [Google Scholar], [Publisher]
[19] A. Qin, A. Musket, P.R. Musich, J.B. Schweitzer, Q. Xie, Receptor tyrosine kinases as druggable targets in glioblastoma: Do signaling pathways matter?, *Neuro-Oncology Advances,* **2021**, *3*, 1. [Crossref], [Google Scholar], [Publisher]
[20] Z. Du, C.M. Lovly, Mechanisms of receptor tyrosine kinase activation in cancer, *Molecular Cancer,* **2018**, *17*, 58. [Crossref], [Google Scholar], [Publisher]
[21] N.J. Szerlip, A. Pedraza, D. Chakravarty, et al, Intratumoral heterogeneity of receptor tyrosine kinases EGFR and PDGFRA amplification in glioblastoma defines subpopulations with distinct growth factor response, *Proceedings of the National Academy of Sciences*, **2012**, *109*, 3041–3046. [Crossref], [Google Scholar], [Publisher]
[22] F. Perrone, L. Da Riva, M. Orsenigo, M. Losa, G. Jocolle, C. Millefanti, E. Pastore, A. Gronchi, M.A. Pierrotti, S. Pilotti, PDGFRA, PDGFRB, EGFR, and downstream signaling activation in malignant peripheral nerve sheath tumor, *Neuro-Oncology*, **2009**, *11*, 725–736. [Crossref], [Google Scholar], [Publisher]
[23] D. Chakravarty, A.M. Pedraza, J. Cotari, A.H. Liu, D. Punko, A. Kokroo, J.T. Huse, G.A. Bonnet, C.W. Brennan, EGFR and PDGFRA co-expression and heterodimerization in glioblastoma tumor sphere lines, *Scientific Reports*, **2017**, 7, 9043. [Crossref], [Google Scholar], [Publisher]

[24] A.S. Arbab, M.M. Ali, Glioblastoma: Targeting angiogenesis and tyrosine kinase pathways, *Novel Approaches in Cancer Study,* **2020**, *4*, 398–401. [Google Scholar], [Publisher]
[25] J.A. Tuszynski, The bioelectric circuitry of the cell, *Brain and Human Body Modeling*, **2019**, *1*, 195–208. [Crossref], [Google Scholar], [Publisher]
[26] Z. Shen, J. Beckel, W.C. de Groat, C. Tai, Effect of high-frequency membrane potential alternation between depolarization and hyperpolarization on dorsal root ganglion neurons of rats, *Physiological Reports*, **2023**, *11*, 2. [Crossref], [Google Scholar], [Publisher]
[27] A.I. Omar, Tumor treating field therapy in combination with bevacizumab for the treatment of recurrent glioblastoma, *Journal of Visualized Experiments* **2014**, *92*, 1. [Crossref], [Google Scholar], [Publisher]
[28] A.M. Davies, U. Weinberg, Y. Palti, Tumor treating fields: a new frontier in cancer therapy, *Annals of the New York Academy of Sciences,* **2013**, *1291*, 86–95. [Crossref], [Google Scholar], [Publisher]
[29] C. Chen, H. Xu, K. Song, Y. Zhang, J. Zhang, Y. Wang, X. Sheng, L. Chen, Z. Qin, Tumor treating fields combine with temozolomide for newly diagnosed glioblastoma: A retrospective analysis of chinese patients in a single center, *Journal of Clinical Medicine,* **2022**, *11*, 5855. [Crossref], [Google Scholar], [Publisher]
[30] F. Winkler, M. Osswald, W. Wick, Anti-Angiogenics: Their role in the treatment of glioblastoma, *Oncology Research and Treatment,* **2018**, *41*, 181–186. [Crossref], [Google Scholar], [Publisher]
[31] H. Bai, J. V. Forrester, M. Zhao, DC electric stimulation upregulates angiogenic factors in endothelial cells through activation of VEGF receptors, *Cytokine,* **2011**, *55*, 110–115. [Crossref], [Google Scholar], [Publisher]
[32] McLendon, Comprehensive genomic characterization defines human glioblastoma genes and core pathways, *Nature,* **2008**, *455*, 1061–1068. [Crossref], [Google Scholar], [Publisher]
[33] A. Oprita, S.-C. Baloi, G.-A. Staicu, O. Alexhandru, D.E. Tache, S. Danoiu, E.S. Micu, A.S. Sevastre, Updated insights on EGFR signaling pathways in glioma, *International Journal of Molecular Sciences,* **2021**, *22*, 587. [Crossref], [Google Scholar], [Publisher]
[34] M.L. Uribe, I. Marrocco, Y. Yarden, EGFR in cancer: Signaling mechanisms, drugs, and acquired resistance, *Cancers,* **2021**, *13*, 2748. [Crossref], [Google Scholar], [Publisher]
[35] M. Hermanson, K. Funa, M. Hartman, L.C. Welsh, C.H. Heldin, B. Westermark, M. Nister, Platelet-derived growth factor and its receptors in human glioma tissue: expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops, *Cancer Research,* **1992**, *52*, 3213–3219. [Google Scholar], [Publisher]
[36] A.M. Cimpean, I.M. Cobec, R.A. Ceausu, R. Popescu, A. Tudor, M. Raica, Platelet Derived Growth Factor BB: A "Must-have" Therapeutic Target "Redivivus" in Ovarian Cancer, *Cancer Genomics Proteomics*, **2016**, *13*, 511–518. [Crossref], [Google Scholar], [Publisher]
[37] E.H. Kim, H.S. Song, S.H. Yoo, M. Yoon, Tumor treating fields inhibit glioblastoma cell migration, invasion and angiogenesis, *Oncotarget,* **2016**, *7*, 65125–65136. [Crossref], [Google Scholar], [Publisher]
[38] J.Y. Oh, Y.J. Lee, E.H. Kim, Tumor-treating fields inhibit the metastatic potential of osteosarcoma cells, *Technol Technology in Cancer Research & Treatment,* **2020**, *19*, 153303382094748. [Crossref], [Google Scholar], [Publisher]
[39] O. Rominiyi, A. Vanderlinden, S.J. Clenton, C. Bridgewater, Y.A. Tamimi, S.J. Collis, Tumour treating fields therapy for glioblastoma: Current advances and future directions, *British Journal of Cancer*, **2021**, *124*, 697–709. [Crossref], [Google Scholar], [Publisher]
[40] G. Olantunji, N. Aderinto, T. Adefusi, E. Kokori, O. Akinmoju, I. Yusuf, T. Olusakin, M.A. Muzammil, Efficacy of tumour-treating fields

therapy in recurrent glioblastoma: A narrative review of current evidence, *Medicine (Baltimore),* **2023**, *102*, 48. [Google Scholar], [Publisher]

[41] V.S. Soni, T.K. Yanagihara, Tumor treating fields in the management of Glioblastoma: opportunities for advanced imaging, *Cancer Imaging* **2019**, *19*, 76. [Crossref], [Google Scholar], [Publisher]

[42] C. Trejo-Solís, N. Serrano-Garcia, Á. Escamilla-Ramírez, R.A.C. Rodriguez, D.J. Farfan, G. Palencia, M. Calvillo, M.A.A. Lemus, A.F. Najera, A.C. Salgado, J. Sotelo, Autophagic and apoptotic pathways as targets for chemotherapy in glioblastoma, *International Journal of Molecular Sciences,* **2018**, *19*, 3773. [Crossref], [Google Scholar], [Publisher]

[43] H. Wang ,Y. Yin , W. Li, X. Zhao, Y. Yu, J. Zhu, Z. Qin, Q. Wang, K. Wang, W. Lu, J. Liu, L. Huang, Over-expression of PDGFR-β promotes PDGF-induced proliferation, migration, and angiogenesis of EPCs through PI3K/Akt signaling pathway, *PlosOne*, **2022**, *7*, 2. [Crossref], [Google Scholar], [Publisher]

[44] I. Nazarenko, S.X. He, A. Hedren, J. Thompson, M.S. Lindstrom, M. Nister, PDGF and PDGF receptors in glioma, *Upsala Journal of Medical Sciences*, **2012**, *117*, 2. [Crossref], [Google Scholar], [Publisher]

[45] A. Jones, T. Schanel, M. Rigsby, C.E. Griguer, B.C. McFarland, J.C. Anderson, C.D. Willey, A.B. Hjelmeland, Tumor treating fields alter the kinomic landscape in glioblastoma revealing therapeutic vulnerabilities, *Cells*, **2023**, *12*, 2171. [Crossref], [Google Scholar], [Publisher]

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