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REVIEW ARTICLE

The Phenomenon of Multidrug Resistance in Glioblastomas

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Abstract

The most common and aggressive brain tumor in the adult population is glioblastoma (GBM). The lifespan of patients does not exceed 22 months. One of the reasons for the low effectiveness of GBM treatment is its radioresistance and chemoresistance. In the current review, we discuss the phenomenon of multidrug resistance of GBM in the context of the expression of ABC family transporter proteins and the mechanisms of proliferation, angiogenesis, and recurrence. We focused on the search of molecular targets among growth factors, receptors, signal transduction proteins, microRNAs, transcription factors, proto-oncogenes, tumor suppressor genes, and their single-nucleotide polymorphisms.

Keywords: Angiogenesis, Apoptosis, Chemotherapy, Glioblastoma, Growth factors, MDR, microRNA, Oncogenes, Proliferation, Tumor suppressor genes

1. Background

Glioblastoma (GBM) is the most common high-grade, chemoresistant and radioresistant brain tumor in the adult population [1]. A life expectancy of patients averages 22.3 months, while 100% have relapses [2]. Malignant tumors, which include GBM, consist of cell populations with different sensitivity to drugs as a result of the therapy undergoing a clonal selection to increase radioresistance and chemoresistance. When such a scenario is realized, a tumor recurs, containing radioresistant and chemoresistant cells. Multidrug resistance (MDR) of tumors is based on the dysregulation of physiological, pathological, and genetic processes that regulate the expression of target genes, tumor suppressors, and proto-oncogenes. These processes involve molecules such as growth factors, cytokines, receptors, signaling pathway molecules, and microRNAs. By contrast, arising changes in DNA and single-nucleotide polymorphism (SNP) contribute to the translation of altered proteins in cells. In this review, we highlight the relationship between proliferation, angiogenesis, metastasis, recurrence, as well as mutations and SNPs in oncogenes and tumor suppressor genes in MDR of GBM.

2. Drug resistance mechanisms

2.1. Drug resistance and transporter proteins of the ABC family

Protein transporters of the ATP-binding cassette (ABC) family of drugs play a key role in the development of MDR. In humans, 49 genes were identified that encode proteins of ATP-binding transporters, which are divided into seven

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subfamilies: *ABCA, ABCB, ABCC, ABCD, ABCE, ABCF,* and *ABCG* [3]. Most of the genes and proteins involved in MDR of GBM belong to the ABCB, ABCC, and ABCG subfamilies. Of the ABCB family, the most well-studied is ABCB1 (ATP binding cassette subfamily B member 1) protein (P-gp, P-glycoprotein), which is expressed on endothelial cells of the blood–brain barrier (BBB) capillaries and glioma stem cells [4]. P-gp is involved in plasma and cerebrospinal fluid excretion of anticancer drugs, organic cations, carbohydrates, oligosaccharides, proteins, and antibiotics [4]. The variety of substrates of P-gp indicates its participation in the protection from toxins of tumor stem cells. The expression of the *ABCB1* gene is regulated by transcription factors: p53 (tumor protein p53), NF-κB (nuclear factor kappa B subunit), AP-1 (K-box region and MADS-box transcription factor family protein), EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit), c-Jun/JNK (c-Jun N-terminal kinase) and signaling pathway kinases: PI3K/AKT (phosphatidylinositol-4,5-bisphosphate 3-kinase/serine/threonine-specific protein kinase 1), GSK-3β (glycogen synthase kinase 3 beta)/β-catenin, MAPK/Ras (mitogen-activated protein kinase/small G-protein)/ERK1/2 (extracellular signal-regulated kinase 1), and p38MAPK (p38 map kinase) [5,6]. MAPK/ERK1/2 kinase pathway is involved in cell survival, proliferation, and motility, which increases the activity of P-gp, whereas the p38MAPK pathway, which activates cell differentiation and apoptosis, decreases the activity of P-gp [7,8]. It is well known that the activation of the PI3K/AKT/NF-κB pathway facilitates survival, proliferation can also enhance the expression of the *MGMT* gene (O6-methylguanine-DNA methyltransferase), and, consequently, GBM resistance to temozolomide (TMZ). Therefore, it is possible that upon activation of the PI3K/AKT/NF-κB pathway, *ABCB1* will participate in the resistance to TMZ.

In the tumor, stem, and BBB cells, overexpression of the *ABCG2* (ATP binding cassette subfamily G member 2) gene and breast cancer–related protein (BCRP) is often observed, enhancing the resistance of GBM to chemotherapeutic agents: vincristine, TMZ, topotecan, and irinotecan [9]. Moreover, co-localization of P-gp and BCRP proteins is observed in GBM and BBB cells, which is associated with their joint functioning as drug transporters [10]. Moreover, BCRP expression is inhibited through suppression of epidermal growth factor receptor (EGFR) expression and activation of PERK (eukaryotic translation initiation factor 2 alpha kinase 3)/ATF4 (activating transcription factor 4)/CHOP (DNA damage-inducible transcript 3) pathways [11].

2.1.1. MicroRNAs

MicroRNAs (miRNAs, miR) are involved in the regulation of the transcription of the *ABCB1* gene and protein translation [12]. For example, miR-200c inhibits the synthesis of P-gp via the JNK signaling pathway [13]. It is possible that miR-130a can activate *ABCB1* expression through PI3K/AKT/PTEN (phosphatase and tensin homolog)/mTOR (mechanistic target of rapamycin kinase) and Wnt/GSK-3β (glycogen synthase kinase 3 beta)/β-catenin signaling pathways [13]. The expression of *ABCG2* is activated by miR-328 and transcription factor EZH2 [14].

2.1.2. Genes ABCB1 and ABCG2

German scientists [15] have studied the effect of three SNPs (rs1128503, rs2032582, rs1045642) in the *ABCB1* gene on the outcome of patients with GBM treated with TMZ. Multivariate analysis showed that the rs1128503 C;C (c.1236 T > C) is a prognostic factor for the survival of patients treated with TMZ. Carriers of C;T and T;T genotypes have 8% and 10% 2-year overall survival (OS) compared with 37% survival in patients with C;C genotype ($p = 0.02$). These data may indicate the role of rs1128503 (*ABCB1*) in the development of resistance of GBM cells to TMZ. The effect of another SNP was identified in *ABCG2* on disease-free and OS in patients ($n = 580$) with colorectal cancer treated with oxaliplatin therapy [15]. It was found that patients carrying *ABCG2* rs2231142 C;C have a lower OS (odds ratio [OR] = 0.666, 95% confidence interval [CI]: 0.527–0.843, $p = 0.001$) than those carrying C;A and A;A genotypes [16].

2.2. Drug resistance and proliferation

The proliferation of GBM cells is induced by aberrant activation of EGF/EGFR, PDGF/PDGFRA -B (platelet-derived growth factor/platelet-derived growth factor receptor subunit A, -B), FGF2 (Fibroblast growth factor)/FGF2R, PI3K/AKT/NF-κB, and STAT3 (signal transducer and activator of transcription 3) signaling pathways, which leads to the development of MDR [17,18]. It is assumed that PDGF/PDGFRA, -B and FGF2/FGF2R signaling are linked with the MDR phenomenon. For example, PI3K/AKT pathway increases GBM size [19]. In turn, the expression *TRF2* (telomere repeat-binding factor 2) stimulates the resistance to TMZ [20]. At the same time, MGMT, ROBO1 (roundabout guidance receptor 1) proteins regulate the transcription factors ZEB1 (zinc finger E-box binding homeobox 1) and STAT3, which activate the proliferation and MDR [21]. An activation of MGMT and resistance of

U87MG and U251MG GBM cells to TMZ are also observed during the formation of the complex of β-catenin with GSK-3β as a result of a decrease in the level of β-catenin in the nucleus upon stimulation of the transcription factor FOXO3a (forkhead box O3A) [21].

2.2.1. MicroRNAs

The proliferation of gliomas is activated by miR-9, miR-21, miR-130a, miR-130b, and miR-223 [22,23]. In turn, expression of miR-21 stimulates the passage of the G0/G1 cell cycle and inhibition of the apoptotic gene *PDCD4* (programmed cell death 4), which causes the resistance to doxorubicin [22]. MiR-223 activates GBM progression and its resistance to TMZ through the PAX6 (paired box 6) regulation [23]. Drug resistance of GBM may be associated with the inhibition of miR-218, miR-328, and Let-7f (microRNA let7f) expression. Let-7f inhibits the expression of oncogenes via the Let-7f/Pereostin pathway. This suppresses proliferation, migration, and invasion of GBM [24,25].

2.2.2. EGF, EGFR, MGMT, GSTP1, and IDH1/2 genes

An activation of signal transduction pathways induces the overexpression of the genes *MGMT*, glutathione S-transferase π1 (*GSTP1)* which trigger the proliferation and chemoresistance [26]. The participation of these genes in GBM progression is confirmed by clinical genetic studies in which progression-free survival (PFS) and OS in patients with rs16950 (c.313A > G) A;A genotype (*GSTP1*) treated with TMZ were 10.5 months (95% CI: 6.7−14.4) and 14.3 months (95% CI: 10.7−17.9), respectively (Table 1) [26]. In addition, GBM progression is also associated with the presence of the rs4444903 (c.61A > G) A allele (OR = 1.30, 95% CI: 0.91−1.87, p = 0.037) and A;A genotype (OR = 1.76, 95% CI: 0.82−3.77, p = 0.037) in the *EGF* gene compared with G;G genotype. Carriers of genotypes A;A (p = 0.042), A;G (p = 0.006), and A;A + A;G (p = 0.008) have a higher level of EGF than carriers of G;G genotype (Table 1) [27]. Amplification of *EGFR* is associated with an increased risk of developing GBM in patients receiving bevazizumab (OR = 2.39, 95% CI: 1.36−4.18, p = 0.007) and shorter OS (p = 0.011) compared with individuals without amplification [28]. The presence of rs730437 (OR = 1.32; 95% CI: 1.05−1.66, p = 0.016) and rs1468727 (OR = 1.31, 95% CI: 1.04−1.65, p = 0.008) *EGFR* variants is also associated with an increased risk of GBM (Table 1) [29,30]. In another study, patients with *MGMT* rs1625649 (c.485C < A) A;A had a higher level of methylation of the gene promoter and lower

Table 1. Polymorphic Variants in Genes Associated with the Risk of Glioblastoma.

Gene	Location	SNP	Allele, genotype, model association with	Gene product	References
EGF	4q25	**rs4444903** (c.61A > G)	A allele vs G;G (OR = 1.30, 95% CI: 0.91−1.87), A;A vs. G;G (OR = 1.76, 95% CI: 0.82−3.77)	Epidermal growth factor	[27]
GSTP1	11q13	**rs1695** (c.313A > G)	A;A, A;G + G;G, (HR = 0.390 (0.196−0.775)	Glutathione S-transferase pi-1	[26]
EGFR	7p11.2	**rs730437** (c.748-49G > A) **rs1468727** (c.1631 + 781C > T)	C;C, C allele (OR = 1.32; 95% CI: 1.05−1.66) C;C, C allele (OR = 1.31, 95% CI: 1.04−1.65)	Epidermal growth factor receptor	[29,30,40]
MGMT	10q26.3	**rs1625649** (c. 485C < A)	A;A vs. C;C, C;A (HR = 2.876, HR = 5.835)	O-6-methylguanine-DNA methyltransferase	[31]
MC4R	18q21.32	**rs489693 (rs1673474)**	A;A vs. A;C/C;C (HR = 3.26, 1.29−8.22)* (HR = 7.02, 2.44−20.2)** *association between SNP and PFS **association between SNP and OS	Melanocortin 4 receptor	[36]
PTEN	10q23.31	**rs7701848** (c.*1516 =)	C;C vs. C;T + T;T (OR = 1.169, 95% CI: 1.061−1.288;53)	Phosphatase and tensin homolog	[38]
VEGFA	6p21-p12, 6p21.1	**rs699947** (c.-2578C > A) **rs1570360** (c.-1154G > A)	C;C + C;A (OR = 2.56 (1.36−4.80) C allele (OR = 1.53 (1.14−2.03) G;G (OR = 1.53 (1.03−2.29), G allele (OR = 1.39 (1.01−1.91)	Vascular endothelial growth factor A	[45]
MTOR	1p36.22	**rs143119651** (c.7496A > G, c.6248A > G, c.6815A > G)		Mechanistic target of rapamycin kinase	[46]
GFAP (ALXDRD)	17q21.31	**rs11558961** (c.*28C > G, c.*28C > A)	G allele (OR = 0.77, 95% CI: 0.61−0.97 C;G (OR = 0.68, 95% CI: 0.49−0.95)	Glial fibrillary acidic protein	[56]

Note. SNP = single-nucleotide polymorphism.

expression of the MGMT protein, which was associated with higher PFS than in patients with heterozygous (C;A) or wild-type (C;C) rs1625649 variants [31].

2.3. Drug resistance and recurrence

The recurrence of gliomas is accompanied by the overexpression of EGFR and PDGFR, the activation of G-protein-coupled receptor kinase 5 (GRK5), 44-fold increase in the expression of SphK1-2 and S1P1-3 compared with normal brain tissue [32]. Constitutive activation of EGFR stimulates hyperactivation of JAK2 (Janus kinase 2)/STAT3 and HGF/c-MET, WNT1-3a/β-catenin signaling pathways which activate the aggressiveness, the rate of proliferation of glioma cells, and activation of telomerase reverse transcriptase (TERT) [33,34]. This correlation is confirmed by the combined use of the EGFR antagonist, Iressa, with the JAK2/STAT3 inhibitor, JSI-124, which causes the death of GBM cells expressing EGFR [33]. EGFR/PTEN/AKT pathways and TGF-β2 pathways also play a role in the malignancy of gliomas [35]. For example, an increase in plasma TGF-β2 levels in patients with GBM is associated with immunosuppression, loss of immune control over tumor progression, and poor prognosis [35].

2.3.1. EGFR, TGFB1, and PTEN genes

It is known that melanocortins have anti-inflammatory and neuroprotective activity. Interestingly, patients with rs489693 (A;A) in the melanocortin-4 receptor (*MC4R*) gene who receive chemotherapy and radiotherapy have shorter PFS (2.99 vs. 10.8 months, $p = 0.009$) and OS (10.8 vs. 29.5 months, $p = 0.0001$) than carriers of A;C and C;C genotypes (Table 1) [36]. An international team of authors from the United States, Canada, Germany, Great Britain, and Israel [37], when carrying out genome-wide association studies with the participation of 12,496 GBM patients and 18,190 healthy controls, established the correlation of *EGFR* rs723527 (c.88 + 47814A > C) with progression of gliomas to GBM (Table 1). However, according to the meta-analysis [38], *PTEN* variant rs701848 (c.*1516 T > C) under the recessive model (C;C vs. C;T + T;T) is associated with the development of glioma, stomach, breast, and endometrial cancer in Asian populations.

2.4. Drug resistance and angiogenesis

During the formation of blood and lymphatic vessels, the stroma and tumor cells exchange secretory molecules. Vasculatory endothelial growth factor receptor 1 (VEGFR1) is strongly expressed, and vasculatory endothelial growth factor receptor-2 and −3 (VEGFR2, VEGFR3) are weakly expressed on GBM cells. The co-localization of MGMT with VEGFR1 receptor activates the PI3K/AKT/mTOR pathway, which enhances invasion, proliferation, migration, and MDR of GBM cells [39]. Stimulation of VEGF/VEGFR, PI3K/AKT/mTOR, and PDGFR pathways in GB9B cells is associated with inhibition of apoptosis and activation of caspases-3, −9, and −8. This is confirmed by the use of selective inhibitors SU1498 for VEGF/VEGFR, BEZ235 for PI3K/AKT/mTOR, and AG1433 for PDGFR, which enhance caspase activation [40]. In GBM cells, PDGF-C (platelet-derived growth factor C) and c-MET kinases are coexpressed. The latter triggers the HGF/c-MET/AKT pathway and enhances MDR [41]. The PI3K/AKT and Hedgehog pathways induce the expression of FGF and its receptor (FGFR), which promotes tumor angiogenesis and MDR [42]. Transactivation of DLL4 (delta-like canonical Notch ligand 4)/NOTCH via FGF2/FGFR and EphB4 (EPH receptor B4)/EprinB2 (ephrin B2) pathways stimulates GBM resistance to the VEGF inhibitor bevacizumab [43]. This pathway involves CDK5 (cyclin-dependent kinase 5), which activates the intracellular domain (NICD) of NOTCH (Neurogenic locus notch homolog) and enhances the growth of lymphatic vessels [44].

2.4.1. VEGFA and MTOR genes

VEGFA rs699947 C allele (OR = 1.53, 95% CI: 1.14−2.03, $p = 0.004$), G allele (OR = 1.39, 95% CI: 1.01−1.91, $p = 0.04$) and rs1570360 G;G genotype (OR = 1.53, 95% CI: 1.03−2.29, $p = 0.03$) are associated with an increased risk of developing GBM (Table 1) [45]. In turn, pathogenic *MTOR* variant rs143119651 (c.7496A > G) may be present in GBM, which reduces the binding of the mTOR protein to its inhibitor Deptor, thereby increasing the resistance of the GBM to environmental deprivation (Table 1) [46]. Since during angiogenesis the PI3K/AKT/mTOR pathway is activated by the VEGFR1 receptor, in the presence of *MTOR* rs143119651, GBM cells can acquire drug resistance.

2.5. Drug resistance and cell death

MDR of GBM is associated with inhibition of apoptosis and JNK/SAPK (mitogen-activated protein kinase 9)/AP-1, NOTCH/HES1 (hairy and enhancer of split-1) pathways, and regulation of genes *CJUN* and *CFOS* [47]. In $CD133^{+}$-GBM cells, the activation of heat shock protein 27 and heat shock protein 72, caspases -9 and -3 are inhibited

through the p38MAPK/MAPKAPK2 (mitogen-activated protein kinase-activated protein kinase 2) pathway that indicates its participation in the development of MDR [48,49]. Since TMZ is known to stimulate the activation of caspase-8, AIF (apoptosis-inducing factor), MAP3K14 (mitogen-activated protein kinase 14), and apoptosis in GBM cells, at the same time, it inhibits the expression of matrix metalloproteinase-9 (MMP-9) and matrix metalloproteinase-2 (MMP-2), which indicates the involvement of MMP-9 and MMP-2 in the development of MDR. The expression of p53 is inversely correlated with MGMT in GBM cells. This indicates the involvement of p53 in tumor sensitivity to TMZ, which is confirmed by the stimulatory effect of carnosol (CAR) on p53, as a result of which the p53-MDM2 complex dissociates, the p53 protein content increases, and its synergistic effect with TMZ on GBM cells is manifested [50].

2.5.1. MicroRNAs

MicroRNAs regulate the transcription of apoptotic genes. For example, overexpression of miR-497 enhances glioma resistance to TMZ through suppression of the tumor suppressor PDCD4 [51]. Overexpression of miR-4284, activation of miR-183/96/182 microRNA cluster, expression of AKT-dependent genes *FGF9* and FOXO1 (Forkhead box O1) are observed in GBM stem cells. At the same time, an inhibition of the microRNA cluster induces ROS-dependent apoptosis via the p53 pathway [52]. MicroRNAs: miR-125b, miR-34a, miR-504, miR-380-5P, miR-885-5P, miR-145, miR-34a for wild type p53, and miR-21 regulate the expression of the p53 protein. MiR-21 also regulates other apoptotic proteins: BCL-2 (B cell leukemia/lymphoma 2), caspase-9, and BAX (BCL2-associated X, apoptosis regulator), the latter is also regulated by miR-222 and miR-34a [53].

2.5.2. Genes TP53 and GFAP

The presence of mutations in gene *TP53* and activation of BCL-2 lead to suppression of the activity of apoptotic proteins (caspase-3 and FADD [Fas-associated death domain]), increased angiogenesis, and cell resistance to cisplatin [54]. For example, an AAG deletion (c.594_597del) (rs1555524949) of the *TP53* gene, leading to a deletion of Glu199 in the p53 protein, is associated with rapid tumor progression [55]. Interestingly, G allele (OR = 0.77, 95% CI: 0.61−0.97, $p = 0.042$) and rs11558961 C;G genotype (c.*28 =) of the *GFAP* gene encoding the glial fibrillar acidic protein (OR = 0.68, 95% CI: 0.49−0.95, $p = 0.022$) are associated with a decrease in GBM chemoresistance (Table 1) [56].

3. Conclusions

Chemoresistance is associated with aberrant effects of growth factors, cytokines, dysregulation of their receptors' activity, signaling transduction pathways, the presence of mutations and SNPs in oncogenes, and tumor suppressor genes involved in proliferation, angiogenesis, cell metastasis, differentiation, apoptosis of GBM cells. The expression of ABC transporters in GBM is regulated by miR-130a, miR-200c microRNAs, transcription factors NF-κB, AP-1, phosphatases, and kinases PTEN, PERK, GSK-3β. With the proliferation and MDR of GBM are associated signaling pathways: EGF/EGFR, FGF2/FGF2R, PDGF/PDGFRA-B, STAT3, transcription factors ZEB1, ROBO1, FOXO3a. The presence of polymorphisms rs1695 *GSTP1,* rs4444903 *EGF,* rs730437, rs1468727, and rs723527 *EGFR* is associated with the activation of cells proliferation of GBM. Angiogenesis and MDR are stimulated by the signaling pathways VEGF/VEGFR1-3, PI3K/AKT/mTOR, and EGFR/JNK/ERK1/2. Polymorphisms rs699947, rs1570360 (*VEGFA*), and rs143119651 (*MTOR*) are associated with both angiogenesis and MDR of GBM cells. In the recurrence of GBM are involved signaling pathways EGF/EGFR/JNK/ERK1/2/AP-1, JAK2/STAT3, HGF/c-MET, WNT1-3a/β-catenin, EGFR/PTEN/AKT; genes *EGFR, TGFB1,* and *PTEN* can be associated with the progression of GBM. Apoptosis and MDR are associated with JNK/SAPK/AP-1, NOTCH/HES1, p38MAPK/MAPKAPK2, and MAP3K14 signaling pathways, proapoptotic proteins AIF, BCL-2, CASP8, and p53, and genes *CFOS* and *CJUN.* Inhibition of apoptosis is associated with rs11558961 (*GFAP*) polymorphism. Thus, the aforementioned factors can be considered as targets in the development of new targeted antitumor biological and chemotherapy drugs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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