Chapter 4

Genetic Alterations of Glioblastoma

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Additional information is available at the end of the chapter

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Abstract

The current research in oncology is focused on genetics and molecular oncology in order to obtain better understanding of the etiology of tumor disease. Detailed knowledge of oncogenesis mechanisms could lead to invention of effective therapeutic tools against cancer. Under healthy conditions, cell cycle is regulated by oncogenes and tumor suppressor genes, which should be in strict balance. Genesis of tumor is a consequence of the accumulation of genetic alterations, which help the cell to escape normal cellular regulatory mechanisms and destruction by immune system. Glioblastoma (GBM) is a highly malignant primary brain tumor occurring mostly in population of adults. Patients suffering from GBM have very poor prognosis. Despite development in radiology methods promising earlier diagnosis and development of clinical and radiation oncology with newer treatment regimes, the effect of therapy remains limited and prognosis of patients has not improved as expected. Target of GBM research are genes involved in response to oxidative stress and DNA damage, genes regulating cell cycle, genes determining immune response, growth factors, and others. Genetic alterations are studied in connection to their possible relationship to susceptibility of brain tissue for tumor formation, to sensitivity of brain tissue for various environmental etiology factors, to effect of anticancer treatment or resistance of tumor tissue to therapy, to overall survival, and progression-free interval.

Keywords: glioblastoma, genes, alteration, mutations, genetic pathways

1. Introduction

Glioblastoma (GBM) is a brain tumor of neuroectodermal origin. It is a second most common primary brain neoplasm and the most common from malignant brain tumors. This tumor arises from neural stem cells (NSCs), progenitor cells, dedifferentiated mature neural cells or neuroepithelial stem cells that transform into cancer stem cells (CSCs) or glioblastoma stem (GSC) or stem-like cells [1]. Stem cells have a high potential of self-renewal and differentia-

tion. GBM is a tumor with highest degree of anaplasia within gliomas. It is classified as grade IV according to the WHO classification of brain tumors from 2007. This lesion has a rapid growth, is unbounded, infiltrates surrounding brain tissue, but rarely metastasizes. When metastases occur, it is usually within the central nervous system. Typical histopathological features of this tumor are cells of recognizably astrocyte origin, but displaying cellular pleomorphism with multinucleation, frequent mitoses, and areas of necrosis surrounded by palisading nuclei (increased tumor cell density) and endothelial proliferation as a manifestation of cellular hyperplasia with numerous clusters of blood vessels forming so-called glomeruloid formations. GBM cells spread along tracts in white matter infiltrate cerebrospinal fluid and vessels between meningeal layers. Invasion of GBM cells begins with the degradation of surrounding matrix proteins by proteases and proteinases. Movement of tumor cells through surrounding brain tissue requires receptor turnover, formation and degradation of focal adhesion molecules and rearrangement of cytoskeleton components. These changes are consequences of genetic alterations, such as overexpression, amplification, deletion or mutation in focal adhesion kinase and phosphatidylinositol 3-kinase (PI3K) pathways [2] and mainly caused by activation of growth factors and their receptors (integrins and protein deleted in colorectal cancer (DCC), hyaluron receptors CD44, RHAMM, BEHAB, ontogenetic protein SPARC, receptors for platelet-derived growth factor (PDGF), transforming growth factor (TGF)-α, epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF)). Some components of extracellular matrix such as laminin or fibronectin are overexpressed in GBM and their silencing reduces invasiveness of GBM cells [3]. Besides microscopic characteristics, GBM is connected with a huge amount of genetic alterations causing higher proliferation, migration, and invasiveness of tumor cells. The intrinsic ability of GBM cells to invade normal brain tissue impedes complete surgical resection and predictably results in early local recurrence and mortality. Thoroughgoing explanation of GBM genetic pathways with their gene alterations is necessary for choosing of more suitable therapy and in predicting patient prognosis. This chapter offers an overview of the most common genetic alterations occurring in GBM.

2. Classification of GBM

According to the WHO classification of brain tumors based on histopathological origin, GBM is a primary brain tumor of neuroepithelial, glial origin. Ranking of GBM in clinical and prognostic significance is within the highest grade IV. GBM could also be classified according to mode of occurrence, as it could be a result of progression from less malignant glial tumor (so-called secondary type) or it could occur de novo (primary type). Another specific type is pediatric GBM. The most common is primary type. Primary, secondary, and pediatric GBMs have their own specific genetic and epigenetic alterations occurring in their gliomagenesis (see **Figure 1**). As diagnosis of GBM presents with heterogeneity of altered genetic pathways evidenced by The Cancer Genome Atlas Research Network's study, classification based on gene expression profile distinguishes a classical, mesenchymal, proneural, and neural type of GBM. Classical type typically harbors no TP53 mutations, but very high rate of epidermal growth factor receptor (EGFR) mutations. This type has a slightly better survival. Mesenchy-

mal type displays frequent mutations of neurofibromatosis gene 1 (NF1), TP53, and PTEN genes and aggressive anticancer therapy brings a significant increase in survival of these patients. Third, proneural subtype presents with highest mutation rate of TP53, PDGFRA, and isocitrate dehydrogenase (IDH) and usually affects younger adults. Last, neural type occurs more often in older population and contains several gene mutations at an average rate [4]. These classifications show high heterogeneity of genetic profile, aggressiveness, clinical characteristics, and expected prognosis of GBM patients with a strong need of definite uniform classification of subtypes leading to more specific treatment for each GBM subtype.

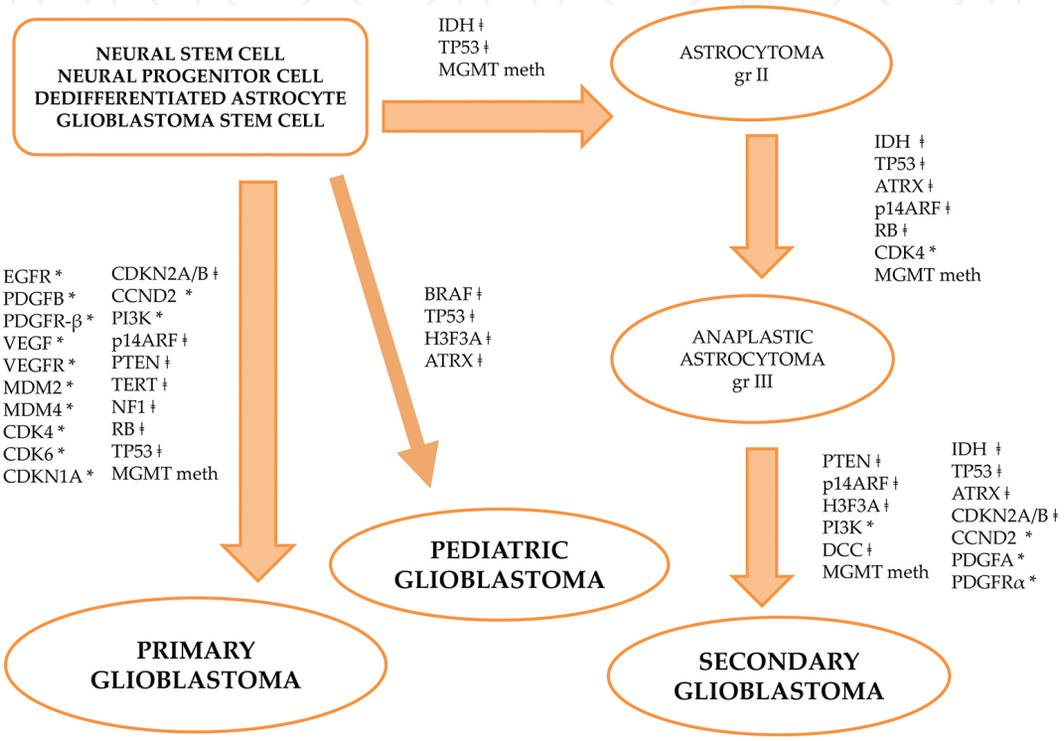

Figure 1. Genes involved in genesis of primary, secondary, and pediatric glioblastoma. *Gain of function through amplification or mutation; ǂhomozygous deletion or mutation.

3. Most frequent genetic alterations of GBM

As mentioned above, alterations of oncogenes and tumor suppressor genes could deflect cell from normal cell cycle. Accumulation of genetic alterations of these genes initiates oncogenesis. In oncogenes, lesion of one allele is sufficient to cause mutation. Activation of oncogene causes avoidance of apoptosis and cell further proliferates. Amplification and activating mutation are the most common genetic alteration of oncogenes. On the other hand, lesion of both alleles is required to evoke mutation in tumor suppressor genes. These genes and their protein products when activated inhibit cell proliferation. Frequently observed alterations are deletion and inhibitory mutation.

During years of genetic research, there have been established three critical genetic pathways whose alterations lead to the formation of GBM: inactivation of p53 and retinoblastoma (RB) pathways (see **Figure 2**), activation of the PI3K pathway, and deregulation of growth factor (receptor tyrosine kinase—RTK) signaling (see **Figure 3**). TP53 signaling pathway is altered in 87% of GBMs, mostly affecting p53, murine double minute-2 (MDM2), MDM4, and cyclin-dependent kinase (CDK)N2A genes. Approximately 78% of GBMs harbor RB signaling disruption with most frequently altered genes: RB1, CDK4, CDK6, CCND2, and cyclin-dependent kinase inhibitor 2 (CDKN2) family. Finally, RTK/RAS/PI3K activation was found in 88% of tumors, affecting usually NF1, PIK3R1, and PIK3CA genes.

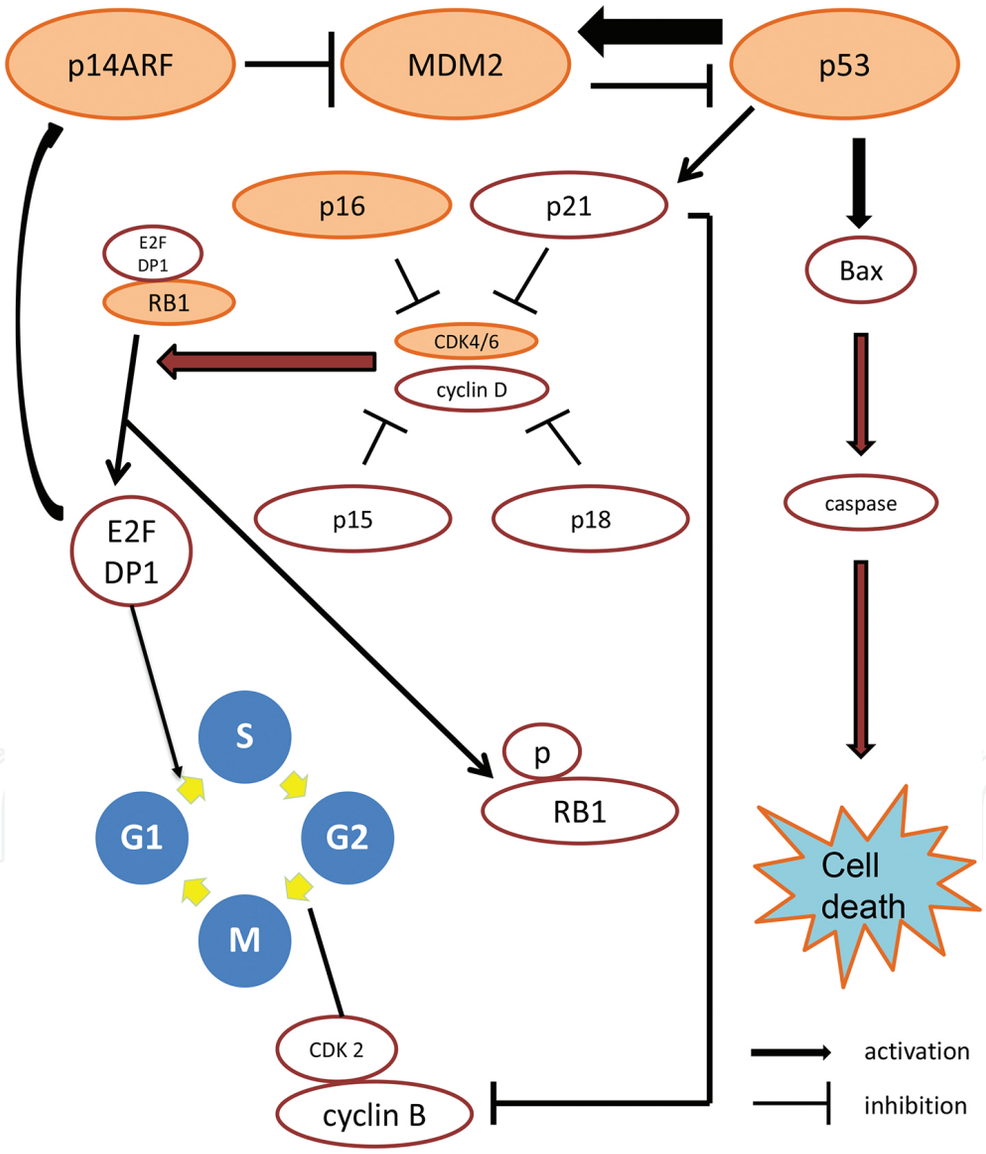

Figure 2. p53 and RB cell cycle and cell death signaling pathway. Proteins marked with full color are most frequently altered in glioblastoma.

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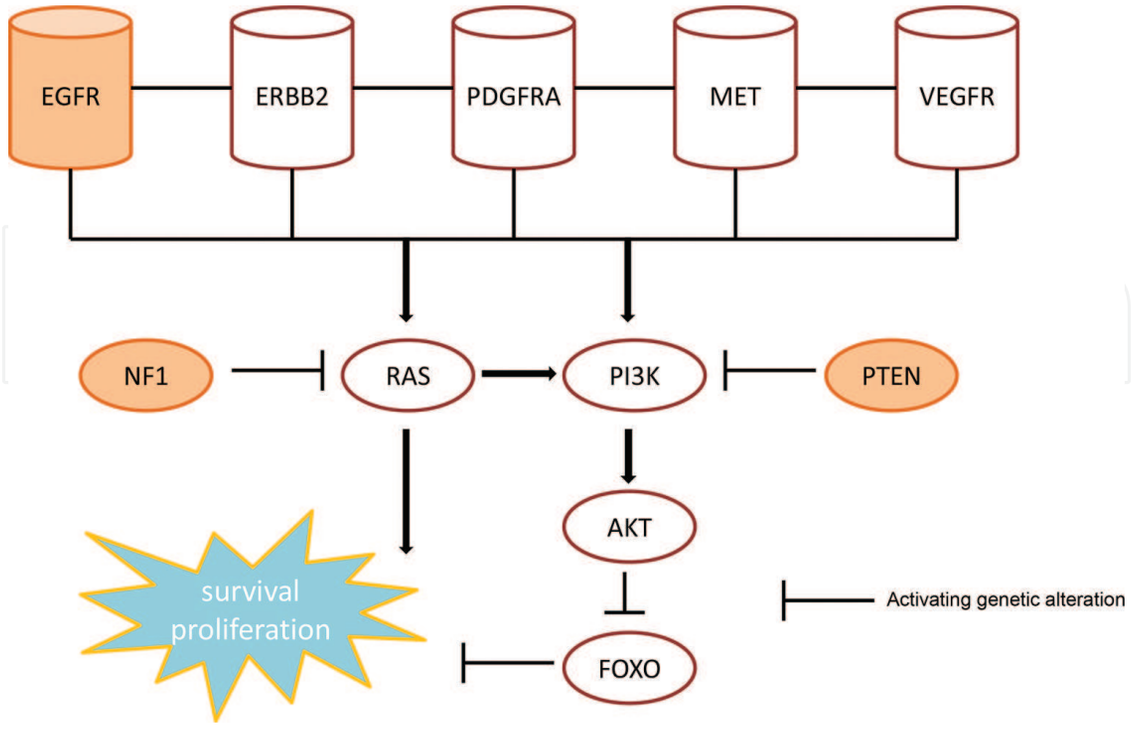

Figure 3. RTK/RAS/PI3K pathway. Proteins marked with full color are most frequently altered in glioblastoma.

GBM is classified as primary or secondary according to altered genes. Primary GBM is a tumor with de novo formation. On the other hand, GBM as a result of malignant transformation of lower-grade glial tumor is called secondary [1]. Also, clinical manifestation and age of diagnosis vary within these two types. Typical for primary GBM is very short anamnesis and age over 50. On the contrary, patients with secondary GBM usually have previous anamnesis of lower grade glioma and younger age depending on the age at the time of diagnosis of preexisting lower grade glioma. Both – primary and secondary GBM – have their own specific gene alterations occurring in their gliomagenesis (see **Figure 1**). Typical alterations for primary GBM are homozygous deletion or mutation of *EGFR*, *PDGFRα*, *MDM2*, *CDK4*, and *PI3K* genes, and amplification or mutation of *CDKN2A/B*, *PTEN*, *NF1*, and *RB* genes. Secondary GBM also has some typical alterations, which are mutation of *IDH* and *TP53* genes and 9p deletion. The best-known prognostic factor of GBM, hypermethylation of methylguanin-O-methyltransferase *(MGMT)* gene promoter, occurs in both primary and secondary GBM.

Gene **TP53** is a crucial tumor suppressor gene located on 17p13.1. It is one of the most frequently mutated genes in human tumor cells [5]. Protein p53 restricts cell proliferation and growth, modulates cell reaction to DNA damage, activates DNA repair, causes cell arrest in G1/S regulation point, and induces apoptosis, senescence, and autophagy. TP53 also regulates neovascularization and cell differentiation [6]. Suppression of pluripotency and inhibition of self-renewal of stem cells belong to the most recently discovered functions of TP53 [7]. TP53-mediated apoptosis is almost not present in glioma cells. Dysfunction of TP53 disrupts the p14ARF pathway, what delays apoptosis and that further promotes genome instability. [8]. High incidence of alterations is in p53-binding domain, mainly codons 175, 248, and 273.

Polymorphism Arg72Pro is under suspicion of higher susceptibility to GBM, but this relationship is not yet definitely confirmed. Mutation or deletion of TP53 is found in 35% of GBM cases, but is more frequent in astrocytomas grade II [6, 9] and plays a role in their progression to secondary GBM. On the other hand, it is present in only about 10% of primary GBM [10].

Oncogene **MDM2** is located on 12q14.3-q15. Protein product of this oncogene is E3 ubiquitin ligase which acts like transcriptional factor. This ligase has five forms and two of them have the ability to interact with p53. The function of this protein is suppression of cell cycle arrest and apoptosis by negative effect on p53 and promotion of cell growth and renewal [11]. The effect of MDM2 is repression of transcriptional activity of p53 and amplification or overexpression of this oncogene completely excludes p53 from having an effect on cell cycle. MDM2 overexpression plays an important role in genesis of glioma, but it seems that they are not involved in glioma progression into higher grade [12]. This overexpression is present in about 10–14% of GBM and is always connected with the presence of wild type TP53. It is an alternative mechanism to disrupt p53 pathway in tumor genesis [6]. This alteration is typical for primary GBM [12].

MDM4 is located on 1q32. MDM4 encodes mdm2-related protein, which has p53-binding domain and RING finger domain. MDM4 binds p53 protein and so inhibits p53-mediated transcriptional transactivation. It also binds MDM2 protein via RING finger domain. Overexpression and amplification of MDM4 occur in GBM, though it is not common (7%). MDM4 alterations do not occur together with p53 or MDM2 alteration. It is an alternative mechanism by which a small part of GBMs escapes p53-regulated growth control. This oncoprotein may inhibit oncogenesis. Ubiquitin-specific protease 2a (USP2A) binds to MDM4, stabilizes it and protects against degradation. USP2–MDM4 interaction could be a determinant of malignant potential. MDM4 and USP2a are highly expressed in GBMs from patients with longer survival [13].

RB gene located on 13q14.2 has a protein product RB, which controls cell cycle, and acts as a gatekeeper to S-phase entry. RB protein is an end point of kinase activities of CDK4/6-cyclin D complexes. Transcription factor E2F binds to RB protein, which is normally not phosphorylated. Complex RB–E2F is a silencing complex restricting access to transcription factors. Phosphorylation of RB leads to release of E2F and such conformation allows CDK2 to access cell cycle during S-phase and this promotes another phosphorylation and that further inhibits the binding of E2F to RB protein [14]. Mutation of this gene has the same effect on cell cycle as the amplification of CDK4/CDK6 or mutation of CDKN2A/CDKN2B, but these alterations are never present together. This means that gliomagenesis proceeds by only one of these alternative genetic pathways. RB pathway is frequently altered in GBM and has a huge role in gliomagenesis [15]. Alterations of RB pathway are present almost universally in human cancers. It is very common in primary GBM, and is present in about 80% [4, 16]. Impaired RB1 expression is associated with increased tumor proliferation and decreased survival; however, more direct correlation with prognosis is in astrocytomas grade II and III than in GBM [17]. Mutation or deletion of RB1 is present in 11% of GBM [16].

CDKN1A gene encodes protein p21 and is located on chromosome 6p. Protein p21 is a member of kinase inhibitor protein family (KIP). Level of p53 protein regulates transcription of p21.

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Apoptosis and cell cycle arrest mediated by p53 induce transcription of p21. This protein controls cell proliferation by regulatory binding to complexes of CDK. It negatively effects complexes of cyclin E/CDK2 and cyclin A/CDK2 and activates cyclin D/CDK. CDKN1A amplification is frequent in GBM [18].

Gene **CDKN1B** is localized on 12p13.1-p12. Its protein product is p27, which is also a member of KIP family. This protein also binds to CDK complexes, inhibits cyclin E/CDK2 and cyclin A/CDK2, and it activates cyclin D/CDK. In proliferating cells, p27 was found in complex with cyclin D/CDK and in G_1 – arrested cells, p27 was bound to cyclin E/CDK2. Very low levels of expression [19] are typical of GBM.

On 9p21 are located genes **CDKN2A** (protein product p16) and **CDKN2B** (product p15). These proteins are inhibitors of CDK and they inhibit phosphorylation of RB1 protein by binding to cyclin D, what prevents their interaction with CDK4/6. Regulation of p16/p14ARF pathway is determining the susceptibility to the only proved environmental risk factor of gliomagenesis – ionizing radiation [20]. Higher risk of glioma formation was observed in patients with CDKN2A and CDKN2B mutations [21, 22]. Homozygous deletion of CDKN2A is present in almost 50% of gliomas and is connected with poor prognosis [14]. But polymorphisms of *CDKN2A*/*CDKN2B* genes are not proved to be connected with tumor grade and prognosis [23].

Protein **p14ARF** is an alternate reading frame product of CDKN2A locus on 9p21. This protein has tumor suppressor activity, regulates cell cycle, initiates p53-dependent cell cycle arrest and apoptosis [20] and is induced by mitogenic stimulation. It inhibits MDM2 and so promotes p53. The finding of p14ARF loss in conjunction with p53 gene loss suggests that the protein may have other p53-independent tumor suppressor functions. P14ARF mediates antiangiogenic effects by upregulating expressions of tissue inhibitor of metalloproteinase-3 in p53-independent fashion. About 60–80% of high-grade gliomas show loss of tumor suppressor p14ARF activity by homozygous mutation [24].

CDKN2C gene has locus on 1p32, encodes protein p18, interacts with CDK4 and CDK6 and prevents their activation. It functions as a cell growth regulator that controls G1 cell cycle progression and also suppresses tumorigenesis. Homozygous deletion is a most common cause of CDKN2C inactivation. This deletion occurs in tumors harboring also CDKN2A deletions. Expression of CDKN2C is almost absent in 43% of GBMs. It is suggested that p16 deletion occurs as an early event in tumorigenesis and p18 inactivation should happen later in tumor formation [25].

Genes **CDK4** (12q14) and **CDK6** (7q21-22) have protein products CDK4 and CDK6 with catalytic kinase activity. Their inhibitors are proteins p15 and p16. CDK4 and CDK6 proteins make complexes with cyclin D. Gene CDK6 is an oncogene, which is required for proliferation of tumor cells and its viability [16]. Overexpression of CDK proteins inhibits the function of their inhibitors – proteins p15 and p16. Amplification of CDK4 is observed in 18% and CDK6 in 1% of GBMs [16].

CCND2 gene (cyclin D2), locus 12p13, encodes a protein of cyclin family that function as regulators of CDK kinases. Cyclin D2 makes a complex with CDK4 and CDK6 and works as a regulatory subunit of this complex, which is required for G1/S cell cycle transition. Associ-

ation of cyclin D2 and CDK4/6 leads to phosphorylation of RB and RB-related proteins that allows cell to enter S phase of cell cycle. Deregulation of this cell cycle transition often causes formation of tumor and is frequently seen in GBM. Amplification of CCND2 occurs in 2% of GBM cases. Study on *in vitro* GSC showed high expression levels of cyclin D2 and suppression of its expression led in experimental model to G1 arrest. This suggests an important role of cyclin D2 in cell cycle progression and in tumorigenicity of GSC [26].

PIK3CA gene (phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha) located on 3q26.3 encodes ATP-dependent IA PI3K alpha catalytic subunit of phosphatidylinositol 3-kinase. This gene is a part of PI3K pathway, which plays a role in proliferation, cellular metabolism, apoptosis, differentiation, migration, and survival [27]. Binding of RTK to its receptor activates regulatory units of PI3K pathway, this activates catalytic subunits, which phosphorylates phosphatidylinositol 4, 5-bisphosphate (PIP2), converts it to phosphatidylinositol 3, 4, 5-triphosphate (PIP3). PIP3 activates downstream signaling cascades as the AKT and mTOR pathways, which are involved in proliferation and cell survival [28]. Hyperactivation of these pathways contributes to tumor progression. Mutations of this gene constitutively activate the PI3K catalytic activity and drive the GBM formation and progression [29]. Treatment of GBM with PI3K-targeted agents is promising [28]. PIK3CB gene (phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit beta) located on 3q22.3 encodes IA PI3K beta catalytic subunit of phosphatidylinositol 3-kinase. This gene was identified as a marker predicting recurrence and prognosis of GBM [30].

PIK3R1 gene (phosphatidylinositol-3-kinase regulatory subunit 1) located on 5q13.1 is also part of PI3K pathway. Mutations and amplifications of these genes also occur in GBM [29]. PIK3R1 mutations act through the catalytic subunit by binding to it and inhibiting its activity [31]. Result of such PIK3R1 mutation is no inhibition of catalytic subunit and so constant activity of whole pathway leading to invasion and migration of tumor cells. Experimental knockdown of PIK3CA and PIK3R1 leads to decrease in proliferation, migration, and invasion of GBM cell lines [32].

Gene phosphatase and tensin homology – **PTEN** located on 10q23.3 is a tumor suppressor gene. PTEN protein, product of this gene, is a lipid phosphatase counteracting the effect of PI3K signaling. PTEN regulates this pathway by converting PIP3 to PIP2. After exposure to ionizing radiation, PTEN acts as a critical determinant of cell fate between the senescence and apoptosis [33]. Germ line mutations of PTEN were described in some autosomal hereditary diseases, such as Cowden disease, Bannayan-Zonana syndrome, etc. Monosomy of chromosome 10 is frequently present in GBM and rarely in low-grade astrocytoma. Inactivation of this tumor suppressor gene is present in both primary and secondary GBM [6]. PTEN gene is mutated or lost in 60–70% of GBM and this condition is associated with poor prognosis [18].

Signal transducer and activator of transcription 3 – **STAT3** gene (locus 17q21.31) encodes a protein member of STAT family. In response to growth factors or cytokines, this protein becomes phosphorylated and acts as a transcription factor. STAT3 regulates cell growth, nervous system development, stem cell differentiation, apoptosis, and is commonly activated in tumors. It also regulates growth and self-renewal of GBM stem cells. STAT3 has an anti-

apoptotic role, is activated in high percentage of GBM, and is required for maintaining of multipotency of tumor cells [34].

Oncogene **AKT3** (v-akt murine thymoma viral oncogene homolog 3) located on 1q44 codes a protein, member of serine/threonine protein kinase family (AKT). These AKT kinases are regulators of processes such as cell proliferation, differentiation, apoptosis, tumorigenesis, glycogen synthesis, and glucose uptake and their overexpression induces cell survival and malignant transformation. Enhanced DNA repair can allow damaged or mutated cells to survive, contributing to resistance and tumor recurrence [35]. Inhibition of AKT kinases activity leads to apoptosis. AKTs have three isoforms AKT1, AKT2, AKT3, being one of the major downstream effectors of PI3K. AKT pathway is activated in 70% of gliomas and is usually associated with PTEN mutations [36]. AKT3 is also essential for normal brain development [37] and is stimulated by PDGF, insulin, and insulin-like growth factor (IGF)1. AKT1 protein does not seem to be overexpressed in GBM, level of AKT2 increases with the malignancy grade and AKT3 expression decreases. It seems that AKT3 protein is affected by the negative feedback caused by AKT2 overexpression. Even though ATK3 is downregulated, it exhibits high kinase activity and retained the functional capacity as an oncoprotein and so may inhibit the growth of malignant cells. AKT3 is often amplified in GBM [35]. AKT2 and AKT3 isoform-specific knockdown inhibits cell growth and induces apoptosis [38] and thus is a potential target for therapy of AKT pathway-altered GBM.

NF1 (locus 17q11.2) codes tumor suppressor protein neurofibromin 1, Ras GTPase-activating protein, which was first found to be mutated in a genetic neurodevelopmental disease neurofibromatosis. Neurofibromin negatively regulates Ras and m-TOR signaling in astrocytes. Approximately 23% of GBM harbor inactivating mutation of NF1, typically mesenchymal subtype. NF1 inactivation could be due to genetic loss or mutation or to extensive proteasomal degradation [39].

MYC oncogene (v-myc avian myelocytomatosis viral oncogene homolog) has locus on 8q24.21. Its protein product is a nuclear phosphoprotein and transcriptional factor with various functions in cell cycle progression, cellular transformation, and apoptosis. Oncogenic activation is achieved by mutation, amplification, overexpression, and chromosomal translocation. This gene is an important target for the cooperative actions of p53 and PTEN in the regulation of normal and malignant cell differentiation, self-renewal, and tumorigenic potential. Overexpression of MYC is present in up to 70% of GBM [40] and surprisingly is highly correlated with better overall survival, what could be a result of enhancement of the proapoptotic effect of upregulated MYC [41]. Moreover, a direct relationship was found between MYC overexpression and MGMT methylation, so that MYC overexpression can be considered a good indicator of response to temozolomide treatment [42].

BMI1 – polycomb ring finger oncogene (10p11.23) is a transcriptional epigenetic repressor of genes governing self-renewal, differentiation, and proliferation, which is directly regulated by MYC. It plays a role in the development of the cerebellum and is required for self-renewal of stem cells in the hematopoietic, epithelial, and nervous system [43]. Overexpression of BMI1 is frequently present in GBM and is concurrent with MYC activation and MYC directly induces upregulation of BMI1 [41, 44].

Isocitrate dehydrogenase system comprises two genes: IDH1 located on 2q33.3 and IDH2 located on 15q26.1. Their products are enzymes (IDH1, IDH2, and IDH3) involved in citric acid cycle. The function of IDHs in this basic metabolic pathway is to catalyze oxidative decarboxylation of isocitrate to α-ketoglutarate and reducing NADP+ to NADPH (NAD+ to NADH in case of IDH3). Crucial effect on interaction of an enzyme with a substrate has arginine 132 (R132) in IDH1 and R172 in IDH2. Mutations of these important areas lead to significant reduction of enzyme activity [45, 46]. Mutations of IDH genes are common in patients with secondary GBM, but rare in primary and pediatric (11%) GBM [47, 48]. The fact that IDH mutations are found in a wide spectrum of histologic tumor types indicates that they occur as an early alteration in tumorigenesis [49]. Only 3–7% of primary GBM shows IDH mutations, but is often found in astrocytoma grade II and III and secondary GBM [50]. Though IDH mutations in primary GBM are rare, they create a prognostically favorable subgroup [21]. Also, IDH2 mutation, which is much less frequent than IDH1 mutation, occurs in gliomas [51], but only in about 6% of secondary GBMs [52]. There is also a possible association between the presence of IDH1 mutation and longer overall survival in patients with GBM [53, 54]. Furthermore, IDH mutation appears to be a significant marker of positive chemosensitivity in secondary GBM. Patients with this mutation show a higher response rate to temozolomide, which is widely used in chemotherapy of malignant gliomas. This makes IDH mutation an independent favorable prognostic factor for overall survival with comparable importance to hypermethylation of MGMT promotor [52]. Patients having both IDH mutation and MGMT promoter methylation seem to have best response to therapy and progression-free survival. Mechanism leading to increased effect of chemotherapy is not clearly known, it is possible that IDH mutation promotes treatment-induced apoptosis by inhibiting the cell-protective mechanisms against oxidative stress [55]. Prolonged survival of secondary GBM patients with IDH mutation was proved in patients with tumors of astrocytic, but not oligodendroglial origin [56]. Tumors with IDH mutation often show also the presence of TP53 mutation; on the other hand, tumors with wild type IDH often have alteration typical for primary GBM (*PTEN*, *EGFR*, *CDKN2A*, or *CDKN2B*). This fact confirms the existence of separate genetic pathways for primary and secondary GBM [50]. IDH mutations are novel and very important and useful prognostic factor of gliomas [21]. An inhibitor of IDH1 significantly retards GBM growth through inducing differentiation [57].

DCC gene with locus on 18q21.3 has product DCC protein. This protein is highly expressed in the nervous system. DCC is a transmembrane protein and creates part of the receptor for netrin 1. Netrins play a role in direct cell and axon migration during neural development. Their expression is also detectable in adult tissue, but their function is not yet clear [58]. DCC induces apoptosis and cell cycle arrest in G2 phase. Also plays a role in chemo-attraction to netrin-1 slowing the rate of spontaneous cell migration. DCC has probably anti-oncogenic function, although if disruption of netrin signaling contributes to tumorigenesis is not yet understood [59]. According to immunohistochemical examination, expression of DCC decreases in many malignancies including GBM and also decreases during progression from low-grade astrocytomas to GBM. This points out the connection of DCC with the formation of secondary, but not with primary GBM [59].

Paternally expressed gene 3 – **PEG3**, located on 19.chromosome, is an imprinted gene expressed mainly during embryogenesis, but also in some adult tissues – ovary, testis, muscle, and brain [60]. Overexpression is frequent in oligodendrogliomas, for GBM very low expression is typical. Deregulation of PEG3 induces the formation of gliomas [61].

GST genes – glutathione S-transferases genes: GSTP1 (location 11q13), GSTM1 (1p13.3), GSTT1 (22q11.23), and GSTO1 (10q25.1). Proteins of these genes are phase II biotransformation enzymes, which detoxify a wide range of exogenous agents including carcinogens. GSTs are responsible for glutathion conjugation of alkylators and scavenging of free radicals generated by radiation [62]. These enzymes provide enzymatic and nonenzymatic functions. GSTs also have important roles in cellular processes as cell proliferation, apoptosis, oncogenesis, tumor progression, drug resistance, and cell response to stress [63]. Genetic variations of GST genes determine the response to carcinogens. Polymorphisms of these genes result in absent or altered enzyme activity and are associated with survival rate in cancer patients. The relationship between these polymorphisms and brain tumor risk is not clear, although there have been some results referring to possible association between GSTM1 null genotype and brain tumor incidence [64]. Patients with brain tumors and GST polymorphisms have higher risk of adverse effects of chemotherapy with nitrosourea alkylating agents.

EGF gene is located on 4q25. EGF is a ligand for its receptor (EGFR), inducing a dimerization of one or several members of the EGFR family (ErbB1-4). This activates several tyrosine kinases and other downstream signal molecules promoting transcription in the cell nucleus [65]. These molecules are important for cell proliferation, survival, migration, and differentiation. EGF polymorphism 61G/A on the promoter part of this gene is associated with glioma susceptibility and with the degree of malignancy [66]; however, results of studies are controversial, probably due to diverse distribution of EGF gene frequencies among the different ethnic groups. EGF 61G/A polymorphism is also probably associated with the survival time of glioma patients [67].

EGFR gene, located on 7p12, encodes EGFR protein, which is a transmembrane receptor responsible for communication with its extracellular ligands – EGF and TGF-α and for transmission of their signalization within the cell. EGFR has an effect on cell apoptosis, angiogenesis, tumor proliferation, and ability to metastasize and is connected to sensitivity to therapy and drug resistance. EGFR is the most frequently amplified gene in astrocytomas, mainly in GBM. Expression of EGFR is higher in high-grade and poorly differentiated GBMs. Both overexpression and amplification of EGFR are correlated with tumor progression. Over 50% of GBM have mutations of EGFR. Presence of EGFR mutation is associated with early relapses and poor prognosis [68]. These mutations exist in three variations: EGFRvIII, δEGFR, and de2-7EGFR (deletion, loss of exons 2-7 of mRNA strand). δEGFR promotes tumorigenesis of GBM by increasing cellular proliferation, decreasing apoptosis, and promoting tumor cell invasiveness. Amplification and de2-7EGFR-specific mutation is typical for primary GBM, in which monosomy of chromosome 10 is also simultaneously present. Amplification of EGFR is mostly associated with CDKN2A deletion, but usually occurs without homozygote deletion or mutation of PTEN. Alteration of EGFR alone is probably not sufficient to cause tumorigenesis, but another alteration in RB1 pathway is required.

PDGF exists in five types of dimers consisting of four types of polypeptides, each encoded by a different gene – A, B, C, and D. PDGFA (7p22), PDGFB (22q13.1), PDGFC (4q32), and PDGFD (8q11). This system comprises two receptors: PDGFR-α (gene PDGFRA, 4q11-q13) and PDGFR-β (gene PDGFRB, 5q31-q32). PDGF system is involved in cell cycle regulation, cell migration, and chemotaxis and has also developmental functions – also in glial development [69]. PDGFR-α is expressed only in subset of GBMs. PDGFR-β is more commonly expressed in GBM by self-renewing GBM stem cells. PDGF pathway aberrations are mostly associated proneural and mesenchymal subtypes of GBM [70]. Overexpression of PDGF and its receptors is often found also in low-grade gliomas, suggesting this alteration as an early oncogenic event and if often found in secondary GBM [71].

IGF system is composed of two ligands: IGF1 (gene IGF1, 12q23.2), IGF2 (also called somatomedin A, gene IGF2, 11p15.5), their receptors: IGF1R (15q26.3) and IGF2R (6q26), six binding proteins (IGFBP 1-6), and various IGFBP-related peptides. IGF has a role in regulation of cell processes. IGF1 is a major physiological mediator of the growth hormone and has a huge influence on cell proliferation and differentiation. It inhibits apoptosis by blocking the initiation of the apoptotic pathway. IGF1R is involved in malignant transformation processes. IGF-binding proteins modulate interactions between IGF and IGFR. IGFBP3 (located on 7p13-p12) acts as an apoptotic agent and inhibits the activity of IGF1 and has growth-promoting effects [72]. Amplification of IGF1, IGF2, and IGF1R genes occurs in gliomas and other cancers. No association between IGF polymorphism and GBM risk has been found.

FGF system is a family of factors involved in various cellular processes, such as developmental, mitogen, cytoprotective, and angiogenic functions. System comprises 18 ligands and four FGF receptors and FGF-binding protein, which increases FGF2-dependent proliferation of fibroblast cells and may have an important role in oncogenesis. FGF2 (bFGF, 4q27-q25), known as a pro-angiogenic factor for vessel formation and wound repair, is a potential oncoprotein in GBM. Upregulated FGF2–FGFR1 signaling is implicated in the pathogenesis of GBM. Overexpression of FGF2 promotes GBM cell proliferation and also exerts an anti-apoptotic function by upregulating of anti-apoptotic genes BCL-2 and BCL-X and thus making tumor resistant to chemotherapy [73]. FGF2 also modulates apoptosis and thus promotes survival via resistance to radiation-induced cell death [74].

Vascular endothelial growth factor – **VEGF** system is the most potent angiogenesis- and vasculogenesis-promoting system. There are known six VEGF ligands and three receptors (VEGFR1, locus 13q12, VEGFR2, locus 4q11-q12, VEGFR3, locus 5q34-q35). VEGF system stimulates endothelial cell migration, growth, proliferation, and survival, also acts as microvascular permeability factor and increases extravasation of plasma proteins [75]. Activation of endothelial nitric oxide (NO) synthase leads to generation of NO and thus further activates angiogenesis. The main angiogenic effect of this system is mediated by interaction of VEGF (VEGFA) with VEGFR2. Angiogenesis is crucial for tumor formation, growth, and maintenance of blood supply. As the tumor develops, it may activate secondary angiogenic pathways, such as bFGF, TGF, and PDGF. GBMs exhibit high levels of VEGF [76]. Higher vascular permeability leads also to radiologic postcontrast enhancement. GBMs with high levels of VEGF are more likely to exhibit ring-like enhancement, what makes relatively clear border on

postcontrast imaging. In such tumors, the gross total resection is easier to obtain. High expression of VEGF is associated with poor prognosis, but there are other factors determining prognosis, such as the extent of resection [76].

TGFβ superfamily is involved in morphogenesis, embryonic development, adult stem cell differentiation, immune reaction, wound healing, and inflammation [77]. This superfamily comprises over 30 proteins divided into two branches: TGFβ subfamily and the bone morphogenetic protein-like (BMP-like) group. The TGFβ subfamily contributes to carcinogenesis and makes tumors more aggressive and BMP-like group has a tumor suppression effect [78]. TGFβ subfamily is composed of three isoforms coded by three different genes – TGFBI (19q13), TGFBII (1q41), and TGFBIII (14q24). This subfamily regulates cell proliferation, differentiation, and extracellular matrix production [79]. All three isoforms are widely expressed in GBM, but TGFβ expression is increased in mesenchymal GBM [80].

Proto-oncogene **MET** (7q31) codes hepatocyte growth factor receptor with tyrosine kinase activity and belongs to RTK family. This receptor is usually expressed in epithelial cells. MET after binding with its ligand hepatocyte growth factor (HGF) plays a role in various cellular signaling pathways involved in proliferation, motility, migration, invasion, and tissue homeostasis by activating the RAS, PI3K, STAT, beta-catenin, and NOTCH signal transduction pathways [81]. It is aberrantly activated in many cancers (lung, pancreas, ovary, salivary gland, and breast cancers) as well as in GBM via mutation, amplification, or overexpression. Mutation of MET in GBM is rare [4]. Amplification is present in 5% and overexpression in 13% of GBM [82]. Overexpression and amplification of MET are not perfectly concordant. This abnormal activation of MET promotes malignancy and is partially associated with the aggressiveness as it was found only in grade IV, not in grade II or III gliomas, but relationship to prognosis is not clear [82, 83]. Amplification of EGFR could be associated with aberrant MET expression [4]. Under research are MET inhibitors in GBM treatment. Moreover, targeting the MET pathway potentiates the responsiveness of GBM to γ-radiation [82].

HGF gene (7q21.1) codes for HGF protein, a pleiotropic factor and cytokine, promoting cell proliferation, survival, motility, scattering, differentiation, and morphogenesis [84]. It is usually expressed by mesenchymal cells, but could be secreted also by tumor cells. In addition, HGF has a protective function in several diseases, including liver cirrhosis and promotes tissue regeneration. It also stimulates mobility and invasiveness of tumor cells and induces angiogenesis [81]. GBM with autocrine activation of HGF has also activated MET signaling and that predicts sensitivity to MET inhibitors in treatment [85]. HGF serum levels serve as biomarkers of responsiveness to such therapy. HGF levels in serum and cerebrospinal fluid correlate with prognosis, and are associated with higher mortality and recurrence rates [86].

Gene **ATRX** (α-thalassemia/mental-retardation-syndrome-X-linked), located at Xq21.1, was discovered as a gene responsible for rare developmental disorder characterized by α-thalassemia and intellectual impairment. It also has a role in the regulation of transcription, heterochromatin structure, and genome stability and is frequently deregulated in human cancer [87]. ATRX alterations are commonly present together with IDH and TP53 mutations. They are detected in 80% of secondary GBMs, but only in 7% of primary [88]. Mutations of ATRX gene are frequent also in pediatric GBM [89].

The B-Raf proto-oncogene serine/threonine kinase – **BRAF** gene, located on 7q34, is a member of Raf kinase family and serves as a strong activator of the extracellular signal-regulated kinase/mitogen-activated protein kinase 1 and 2 (Erk 1/2) signal transduction cascade which modulates cell growth, proliferation, migration, differentiation, and apoptosis. Mutations of BRAF gene usually occur at codon 600, which is a site of activation loop of the kinase domain. Consequence of this BRAF V600E mutation is a protein with increased kinase activity. This mutation is usually present in lower-grade gliomas, but was found in 54% of epithelioid GBM [90], in 7% of giant cell GBM [91]. Within classical GBM types, BRAF mutation was present in 8% of adult and in 12% of pediatric GBM cases. These GBMs were all classified as primary. In adults, they create a group of tumors that are present in younger age (mean age of 39 years), none of these tumors harbored IDH mutation and seem to have slightly longer survival [92]. GBMs with BRAF V600E mutation may represent a small, but more favorable subgroup. In such patients, BRAF/MEK inhibitor treatment may be beneficial in combination with other therapies [93].

H3 histone, family 3A – **H3F3A** gene, located on 1q42.12, encodes replication-independent histone H3.3. Histones are basic nuclear proteins responsible for the nucleosome structure of the chromosomal fiber. ATRX and DAXX genes are encoding subunits of a chromatin remodeling complex required for H3.3 incorporation at pericentric heterochromatin and telomeres [94]. The histone H3.3 mutations result in amino acid substitution at K27 or G34 – two critical positions within the histone tail involved in key regulatory posttranslational modifications. H3F3A mutations are often seen in tumors with somatic TP53 mutation, within histological grades are specific for GBM and are highly prevalent in children and young adults, and they are present in one-third of pediatric GBMs [95]. Interestingly, K27-mutated tumors were predominantly seen in midline structures – thalamus, pons (diffuse intrinsic pontine gliomas), and spinal cord and have potentially poor prognosis. G34-mutated GBMs have better overall survival [96].

Telomerase reverse transcriptase –**TERT** gene is located on 5p15.33. This gene encodes the enzymatic core of telomerase. Telomeres are repeated sequences of DNA at the ends of chromosomes. Under physiological conditions, as the cell divides, telomeres become progressively shorter. Telomerase counteracts the shortening of telomeres by adding small segments of DNA at the end of chromosome after each cell division. Enhanced telomerase activity facilitates cellular immortality and promotes oncogenesis. Mutations of TERT promoter unmask binding sites for transcription factors, upregulate TERT expression and cellular telomerase activity [97]. TERT mutations are much more common in primary GBM and are inversely correlated with IDH mutations. Primary GBMs with TERT mutation exhibit significantly shorter overall survival than TERT-wild type tumors [98]. This unfavorable prognosis was absent after gross total resection and temozolomide therapy, what may indicate that TERT mutation makes tumor more susceptible to chemotherapy.

Tumor suppressor **PARK2** gene (parkin RBR E3 ubiquitin protein ligase) has locus on 6q25.2-q27. The exact function of this gene is still to be explored. It codes an E3 ubiquitin ligase mediating the targeting of substrate proteins for proteasome degradation. Germ line mutations of this gene cause Parkinson's disease, somatic mutations contribute to cancer. PARK2 is one

of the most commonly inactivated tumor suppressor genes in GBM [99]. Deletion or inactivating mutation of this gene causes inability of PARK2 to promote ubiquitination and results in cyclin E deregulation, which can promote tumor cell growth. Low expression of PARK2 is associated with poor survival [100].

MGMT gene encodes a protein with alkyltransferase activity. MGMT is a DNA repair enzyme, which is responsible for tumor resistance to alkylating and methylating agents. Activity of this enzyme is regulated by this promoter area. This promoter, when methylated, causes inactivation of MGMT. Approximately 44% of GBM has MGMT promoter methylation [21]. Methylation status of MGMT is clinically the most relevant molecular parameter to predict sensitivity of gliomas do DNA alkylating chemotherapeutics [101]. This methylation is associated with prolonged progression-free survival and overall survival in patients with GBM treated with alkylating agents, such as temozolomide. [102]. Expression of MGMT is not associated with tumor grade [68].

4. Conclusion

GBM is the most aggressive and devastating primary brain tumor with very grim prognosis. The patient's survival rarely exceeds a year and a half with all accessible therapy used. Only 3% of GBM patients have survival over 5 years. This fact makes this tumor a very severe diagnosis directly affecting life expectancy of the patient and quality of his/her life. Explanation of its genesis could bring us closer to invention of effective treatment and genetic profile of concrete GBM tissue would help to design the individualized therapy management for each patient. Understanding the genetic and epigenetic characterization could help to distinguish various GBM subgroups, indistinguishable by histological appearance, but classified according to molecular and genetic alterations. This could lead to establishment of GBM classification with clinical impact, subgroup-specific treatment, and better design of future trials. The aim was to achieve prolonged progression-free interval and overall survival with maintaining satisfactory quality of life. It is very important if concrete genetic alteration is connected to tumor formation or if it is prognostic factor. Other factors influencing prognosis are histological type and tumor grade, age of patient, Karnofsky performance score at the time of diagnosis, extend of surgical resection, tumor localization, and appropriate therapeutic management. Tumor localization and extend of its surgical resection influence progression-free interval as well as overall survival. Detailed and complete explanation and discovery of altered genes and whole genetic pathways of GBM is the basis for new distinctive GBM classification. Such a new tumor division could bring us closer to routine genetic examination of frequently altered genetic pathways from tumor sample and aiming of therapy directly against specific genetic and epigenetic targets. Such individualized therapy could decrease the number of adverse effects, but first of all, hopefully, would finally ameliorate survival and life quality of patients, suffering from this severe disease.

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References

[1] Ricard D, Idbaih A, Ducray F, Lahutte M, Hoang-Xuan K, Delattre JY: Primary brain tumours in adults. Lancet. 2012;379:1984–1996. DOI: 10.1016/S0140-6736(11)61346-9

[2] Michael KE, Dumbauld DW, Burns KL, Hanks SK, Garcia AJ: Focal adhesion kinase modulates cell adhesion strengthening via integrin activation. Mol Biol Cell. 2009;20:2508–2519. DOI: 10.1091/mbc.E08-01-0076

[3] Sengupta SNS, Hindi ES, Wainwright DA, Han Y, Lesniak MS: Short hairpin RNA-mediated fibronectin knockdown delays tumor growth in a mouse glioma model. Neoplasia. 2010;12:837–847.

[4] Cancer Genome Atlas Research Network: Comprehensive genomic characterization defines human GBM genes and core pathways. Nature. 2008;455:1061–1068. DOI: 10.1038/nature07385

[5] Iacob G, Dinca EB: Current data and strategy in GBM multiforme. J Med Life. 2009;2:386–393.

[6] Hede SM, Nazarenko I, Nistér M, Lindström MS: Novel perspectives on p53 function in neural stem cells and brain tumors. J Oncol. 2011;2011:852970. DOI: 10.1155/2011/852970

[7] Zhao Z, Xu Y: p53 and stem cells: new developments and new concerns. Trends Cell Biol. 2010;20:170–175. DOI: 10.1016/j.tcb.2009.12.004

[8] Krakstad C, Chekenya M: Survival signaling and apoptosis resistance in GBMs: opportunities for targeted therapeutics. Mol Cancer. 2010;9:135–149. DOI: 10.1186/1476-4598-9-135

[9] Wade M, Wang YV, Wahl GM: The p53 orchestra: Mdm2 and Mdmx set the tone. Trends Cell Biol. 2010;20:299–309. DOI: 10.1016/j.tcb.2010.01.009

[10] Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C, Chin L, DePinho RA, Cavenee WK: Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev. 2007;21:2683–2710. DOI: 10.1101/gad.1596707

[11] Thomasova D, Mulay SR, Bruns H, Anders HJ: p53-independent roles of MDM2 in NF-κB signaling: implications for cancer therapy, wound healing, and autoimmune diseases. Neoplasia. 2012;14:1097–1101.

[12] Wang AL, Liu ZX, Li G, Zhang LW: Expression and significance of P53 protein and MDM-2 protein in human gliomas. Chin Med J (Engl). 2011;124:2530–2533.

[13] Wang CL, Wang JY, Liu ZY, Ma XM, Wang XW, Jin H, Zhang XP, Fu D, Hou LJ, Lu YC: Ubiquitin-specific protease 2a stabilizes MDM4 and facilitates the p53-mediated intrinsic apoptotic pathway in GBM. Carcinogenesis. 2014;35:1500–1509. DOI: 10.1093/carcin/bgu015

[14] Goldhoff P, Clarke J, Smirnov I, Berger MS, Prados MD, James CD, Perry A, Phillips JJ: Clinical stratification of GBM based on alterations in retinoblastoma tumor suppressor protein (RB1) and association with the proneural subtype. J Neuropathol Exp Neurol. 2012;71:83–89. DOI: 10.1097/NEN.0b013e31823fe8f1

[15] Chow LM, Endersby R, Zhu X, Rankin S, Qu C, Zhang J, Broniscer A, Ellison DW, Baker SJ: Cooperativity within and among Pten, p53, and Rb pathways induces high-grade astrocytoma in adult brain. Cancer Cell. 2011;19:305–316. DOI: 10.1016/j.ccr.2011.01.039

[16] Wiedemeyer WR, Dunn IF, Quayle SN, Zhang J, Chheda MG, Dunn GP, Zhuang L, Rosenbluh J, Chen S, Xiao Y, Shapiro GI, Hahn WC, Chin L: Pattern of retinoblastoma pathway inactivation dictates response to CDK4/6 inhibition in GBM. Proc Natl Acad Sci USA. 2010;107:11501–11506. DOI: 10.1073/pnas.1001613107

[17] Kim CS, Jung S, Jung TY, Jang WY, Sun HS, Ryu HH: Characterization of invading glioma cells using molecular analysis of leading-edge tissue. J Korean Neurosurg Soc. 2011;50:157–165. DOI: 10.3340/jkns.2011.50.3.157

[18] Walker C, Baborie A, Crooks D, Wilkins S, Jenkinson MD: Biology, genetics and imaging of glial cell tumours. Br J Radiol. 2011;84:90–106. DOI: 10.1259/bjr/23430927

[19] Yue WY, Sai K, Wu QL, Xia YF, Yu SH, Chen ZP: Long-term molecular changes in WHO grade II astrocytomas following radiotherapy. Chin J Cancer. 2012;31:159–165. DOI: 10.5732/cjc.011.10149

[20] Liu Y, Shete S, Hosking FJ, Robertson LB, Bondy ML, Houlston RS: New insights into susceptibility to glioma. Arch Neurol. 2010;67:275–278. DOI: 10.1001/archneurol.2010.4

[21] Weller M, Felsberg J, Hartmann C, Berger H, Steinbach JP, Schramm J, Westphal M, Schackert G, Simon M, Tonn JC, Heese O, Krex D, Nikkhah G, Pietsch T, Wiestler O, Reifenberger G, von Deimling A, Loeffler M: Molecular predictors of progression-free and overall. Survival in patients with newly diagnosed GBM: a prospective translational study of the German glioma network. J Clin Oncol. 2009;27:5743–5750. DOI: 10.1200/JCO.2009.23.0805

[22] Shete S, Hosking FJ, Robertson LB, Dobbins SE, Sanson M, Malmer B, Simon M, Marie Y, Boisselier B, Delattre JY, Hoang-Xuan K, El Hallani S, Idbaih A, Zelenika D, Andersson U, Henriksson R, Bergenheim AT, Feychting M, Lönn S, Ahlbom A, Schramm J, Linnebank M, Hemminki K, Kumar R, Hepworth SJ, Price A, Armstrong G, Liu Y, Gu X, Yu R, Lau C, Schoemaker M, Muir K, Swerdlow A, Lathrop M, Bondy M, Houlston RS: Genome-wide association study identifies five susceptibility loci for glioma. Nat Genet. 2009;41:899–904. DOI: 10.1038/ng.407

[23] Simon M, Hosking FJ, Marie Y, Gousias K, Boisselier B, Carpentier C, Schramm J, Mokhtari K, Hoang-Xuan K, Idbaih A, Delattre JY, Lathrop M, Robertson LB, Houlston RS, Sanson M: Genetic risk profiles identify different molecular etiologies for glioma. Clin Cancer Res. 2010;16:5252–5259. DOI: 10.1158/1078-0432.CCR-10-1502

[24] Zerrouqi A, Pyrzynska B, Febbraio M, Brat DJ, Van Meir EG: P14ARF inhibits human GBM-induced angiogenesis by upregulating the expression of TIMP3. J Clin Invest. 2012;122:1283–1295. DOI: 10.1172/JCI38596

[25] Solomon DA, Kim JS, Jenkins S, Ressom H, Huang M, Coppa N, Mabanta L, Bigner D, Yan H, Jean W, Waldman T: Identification of p18 INK4c as a tumor suppressor gene in GBM multiforme. Cancer Res. 2008;68:2564–2569. DOI: 10.1158/0008-5472.CAN-07-6388

[26] Koyama-Nasu R, Nasu-Nishimura Y, Todo T, Ino Y, Saito N, Aburatani H, Funato K, Echizen K, Sugano H, Haruta R, Matsui M, Takahashi R, Manabe E, Oda T, Akiyama T: The critical role of cyclin D2 in cell cycle progression and tumorigenicity of GBM stem cells. Oncogene. 2013;32:3840–3845. DOI: 10.1038/onc.2012.399

[27] Courtney KD, Corcoran RB, Engelman JA: The PI3K pathway as drug target in human cancer. J Clin Oncol. 2010;28:1075–1083. DOI: 10.1200/JCO.2009.25.3641

[28] Wen PY, Lee EQ, Reardon DA, Ligon KL, Alfred Yung WK: Current clinical development of PI3K pathway inhibitors in GBM. Neuro Oncol. 2012;14:819–829. DOI: 10.1093/neuonc/nos117.

[29] Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G,

Vogelstein B, Velculescu VE, Kinzler KW: An integrated genomic analysis of human GBM multiforme. Science. 2008;321:1807–1812. DOI: 10.1126/science.1164382

[30] Varghese RT, Liang Y, Guan T, Franck CT, Kelly DF, Sheng Z: Survival kinase genes present prognostic significance in glioblastoma. Oncotarget. 2016;7:20140-20151. DOI: 10.18632/oncotarget.7917

[31] Sun MHP, Hofmann BT, Hart JR, Vogt PK: Cancer derived mutations in the regulatory subunit p85alpha of phosphoinositide 3-kinase function through the catalytic subunit p110alpha. Proc Natl Acad Sci USA. 2010;107:15,547–15,552. DOI: 10.1073/pnas.1009652107

[32] Weber GL, Parat MO, Binder ZA, Gallia GL, Riggins GJ: Abrogation of PIK3CA or PIK3R1 reduces proliferation, migration, and invasion in GBM multiforme cells. Oncotarget. 201;2:833–849.

[33] Lee JJ, Kim BC, Park MJ, Lee YS, Kim YN, Lee BL, Lee JS: PTEN status switches cell fate between premature senescence and apoptosis in glioma exposed to ionizing radiation. Cell Death Differ. 2011;18:666–677. DOI: 10.1038/cdd.2010.139

[34] Sherry MM, Reeves A, Wu JK, Cochran BH: STAT3 is required for proliferation and maintenance of multipotency in GBM stem cells. Stem Cells. 2009;27:2383–2392. DOI: 10.1002/stem.185

[35] Turner KM, Sun Y, Ji P, Granberg KJ, Bernard B, Hu L, Cogdell DE, Zhou X, Yli-Harja O, Nykter M, Shmulevich I, Yung WK, Fuller GN, Zhang W: Genomically amplified Akt3 activates DNA repair pathway and promotes glioma progression. Proc Natl Acad Sci USA. 2015;112:3421–3426. DOI: 10.1073/pnas.1414573112

[36] Omuro AM, Faivre S, Raymond E: Lessons learned in the development of targeted therapy for malignant gliomas. Mol Cancer Ther. 2007;6:1909–1919.

[37] Easton RM, Cho H, Roovers K, Shineman DW, Mizrahi M, Forman MS, Lee VM, Szabolcs M, de Jong R, Oltersdorf T, Ludwig T, Efstratiadis A, Birnbaum MJ: Role for Akt3/protein kinase Bgamma in attainment of normal brain size. Mol Cell Biol. 2005;25:1869–1878.

[38] Mure H, Matsuzaki K, Kitazato KT, Mizobuchi Y, Kuwayama K, Kageji T, Nagahiro S: Akt2 and Akt3 play a pivotal role in malignant gliomas. Neuro Oncol. 2010;12:221–232. DOI: 10.1093/neuonc/nop026

[39] Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN; Cancer Genome Atlas Research Network: Integrated genomic analysis identifies clinically relevant subtypes of GBM characterized by abnormalities

in PDGFRA, IDH1, EGFR and NF1. Cancer Cell. 2010;17:98–110. DOI: 10.1016/j.ccr.2009.12.020

[40] Faria MH, Gonçalves BP, do Patrocínio RM, de Moraes-Filho MO, Rabenhorst SH: Expression of Ki-67, topoisomerase II alpha and c-MYC in astrocytic tumors: correlation with the histopathological grade and proliferative status. Neuropathology. 2006;26:519–527.

[41] Cenci T, Martini M, Montano N, D'Alessandris QG, Falchetti ML, Annibali D, Savino M, Bianchi F, Pierconti F, Nasi S, Pallini R, Larocca LM: Prognostic relevance of c-Myc and BMI1 expression in patients with glioblastoma. Am J Clin Pathol. 2012;138:390–396. DOI: 10.1309/AJCPRXHNJQLO09QA

[42] De Salvo M, Maresca G, D'agnano I, Marchese R, Stigliano A, Gagliassi R, Brunetti E, Raza GH, De Paula U, Bucci B: Temozolomide induced c-Myc-mediated apoptosis via Akt signaling in MGMT expressing glioblastoma cells. Int J Radiat Biol. 2011;87:518–533. DOI: 10.3109/09553002.2011.556173

[43] Shakhova O, Leung C, Marino S: Bmi1 in development and tumorigenesis of the central nervous system. J Mol Med. 2005;83:596–600.

[44] Guo WJ, Datta S, Band V, Dimri GP: Mel-18, a polycomb group protein, regulates cell proliferation and senescence via transcriptional repression of Bmi-1 and c-Myc oncoproteins. Mol Biol Cell. 2007;18:536–546.

[45] Pusch S, Sahm F, Meyer J, Mittelbronn M, Hartmann C, von Deimling A: Glioma IDH1 mutation patterns off the beaten track. Neuropathol Appl Neurobiol. 2011;37:428–430. DOI: 10.1111/j.1365-2990.2010.01127.x

[46] Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, Yu W, Li Z, Gong L, Peng Y, Ding J, Lei Q, Guan KL, Xiong Y: Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science. 2009;324:261–265. DOI: 10.1126/science.1170944

[47] Toedt G, Barbus S, Wolter M, Felsberg J, Tews B, Blond F, Sabel MC, Hofmann S, Becker N, Hartmann C, Ohgaki H, von Deimling A, Wiestler OD, Hahn M, Lichter P, Reifenberger G, Radlwimmer B: Molecular signatures classify astrocytic gliomas by IDH1 mutation status. Int J Cancer. 2011;128:1095–1103. DOI: 10.1002/ijc.25448

[48] Kim W, Liau LM: IDH mutations in human glioma. Neurosurg Clin N Am. 2012;23:471–480. DOI: 10.1016/j.nec.2012.04.009

[49] Yan H, Parsons DW, Jin G, McLendon R, Rasheed A, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD: IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009;360:765–773. DOI: 10.1056/NEJMoa0808710

[50] Ichimura K, Pearson DM, Kocialkowski S, Bäcklund LM, Chan R, Jones DT, Collins VP: IDH1 mutations are present in the majority of common adult gliomas but rare in primary GBMs. Neuro Oncol. 2009;11:341–347. DOI: 10.1215/15228517-2009-025

[51] Reitman ZJ, Yan H: Isocitrate dehydrogenase 1 and 2 mutations in cancer: alterations at a crossroads of cellular metabolism. J Natl Cancer Inst. 2010;102:932–941. DOI: 10.1093/jnci/djq187

[52] SongTao Q, Lei Y, Si G, YanQing D, HuiXia H, XueLin Z, LanXiao W, Fei Y: IDH mutations predict longer survival and response to temozolomide in secondary GBM. Cancer Sci. 2012;103:269–273. DOI: 10.1111/j.1349-7006.2011.02134.x

[53] Nobusawa S, Watanabe T, Kleihues P, Ohgaki H: IDH1 mutations as molecular signature and predictive factor of secondary GBMs. Clin Cancer Res. 2009;15:6002–6007. DOI: 10.1158/1078-0432.CCR-09-0715

[54] Beiko J, Suki D, Hess KR, Fox BD, Cheung V, Cabral M, Shonka N, Gilbert MR, Sawaya R, Prabhu SS, Weinberg J, Lang FF, Aldape KD, Sulman EP, Rao G, McCutcheon IE, Cahill DP: IDH1 mutant malignant astrocytomas are more amenable to surgical resection and have a survival benefit associated with maximal surgical resection. Neuro-Oncology. 2014;16:81–91. DOI: 10.1093/neuonc/not159

[55] Reitman ZJ, Yan H: Isocitrate dehydrogenase 1 and 2 mutations in cancer: alterations at a crossroads of cellular metabolism. J Natl Cancer Inst. 2010;102:932–941. DOI: 10.1093/jnci/djq187

[56] Takahashi Y, Nakamura H, Makino K, Hide T, Muta D, Kamada H, Kuratsu J: Prognostic value of isocitrate dehydrogenase 1, O6-methylguanine-DNA methyltransferase promoter methylation, and 1p19q co-deletion in Japanese malignant glioma patients. World J Surg Oncol. 2013;11:284. DOI: 10.1186/1477-7819-11-284

[57] Rohle D, Popovici-Muller J, Palaskas N, Turcan S, Grommes C, Campos C, Tsoi J, Clark O, Oldrini B, Komisopoulou E, Kunii K, Pedraza A, Schalm S, Silverman L, Miller A, Wang F, Yang H, Chen Y, Kernytsky A, Rosenblum MK, Liu W, Biller SA, Su SM, Brennan CW, Chan TA, Graeber TG, Yen KE, Mellinghoff IK: An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. Science. 2013;340:626–630. DOI: 10.1126/science.1236062

[58] Rajasekharan S, Kennedy TE: The netrin protein family. Genome Biol. 2009;10:239. DOI: 10.1186/GBM-2009-10-9-239

[59] Jarjour AA, Durko M, Luk TL, Marçal N, Shekarabi M, Kennedy TE: Autocrine netrin function inhibits glioma cell motility and promotes focal adhesion formation. PLoS One. 2011;6:e25408. DOI:10.1371/journal.pone.0025408

[60] Jiang X, Yu Y, Yang HW, Agar NY, Frado L, Johnson MD: The imprinted gene PEG3 inhibits Wnt signaling and regulates glioma growth. J Biol Chem. 2010;285:8472–8480. DOI: 10.1074/jbc.M109.069450

[61] Otsuka S, Maegawa S, Takamura A, Kamitani H, Watanabe T, Oshimura M, Nanba E: Aberrant promoter methylation and expression of the imprinted PEG3 gene in glioma. Proc Jpn Acad Ser B. 2009;85:157–165. DOI: 10.2183/pjab.85.157

[62] Kilburn L, Okcu F, Wang T, Cao Y, Renfro-Spelman A, Aldape KD, Gilbert MR, Bondy M: Glutathione S-transferase polymorphism are associated with survival in anaplastic glioma patients. Cancer. 2010;116:2242–2249. DOI: 10.1002/cncr.25006

[63] Lo H, Ali-Osman F: Genetic polymorphism and function of glutathione S-transferases in tumor drug resistance. Curr Opin Pharmacol. 2007;7:367–374. DOI: 10.1016/j.coph.2007.06.009

[64] Schwartzbaum JA, Ahlbom A, Lönn S, Warholm M, Rannug A, Auvinen A, Christensen HC, Henriksson R, Johansen C, Lindholm C, Malmer B, Salminen T, Schoemaker MJ, Swerdlow AJ, Feychting M: An international casecontrol study of glutathione transferase and functionally related polymorphisms and risk of primary adult brain tumors. Cancer Epidemiol Biomarkers Prev. 2007;16:559–565. DOI: 10.1158/1055-9965.EPI-06-0918

[65] Sjöström S, Anderson U, Liu Y, Brännström T, Broholm H, Johansen C, Collatz-Laier H, Henriksson R, Bondy M, Melin B: Genetic variation in EGF and EGFR and GBM outcome. Neuro-Oncol. 2010;12:815–821. DOI: 10.1093/neuonc/noq018

[66] Chen X, Yang G, Zhang D, Zhang W, Zou H, Zhao H, Zhang X, Zhao S: Association between the epidermal growth factor +61 G/A polymorphism and glioma risk: a meta-analysis. PLoS One. 2014;9:e95139. DOI: 10.1371/journal.pone.0095139

[67] Pinto GR, Yoshioka FK, Clara CA, Santos MJ, Almeida JR, Burbano RR, Rey JA, Casartelli C: Association study of an epidermal growth factor gene functional polymorphism with the risk and prognosis of gliomas in Brazil. Int J Biol Markers. 2009;24:277–281.

[68] Hu X, Miao W, Zou Y, Zhang W, Zhang Y, Liu H: Expression of p53, epidermal growth factor receptor, Ki-67 and O6-methylguanine-DNA methyltransferase in human gliomas. Oncol Lett. 2013;6:130–134.

[69] Ozawa T, Brennan CW, Wang L, Squatrito M, Sasayama T, Nakada M, Huse JT, Pedraza A, Utsuki S, Yasui Y, Tandon A, Fomchenko EI, Oka H, Levine RL, Fujii K, Ladanyi M, Holland EC: PDGFRA gene rearrangements are frequent genetic events in PDGFRA-amplified GBMs. Genes Dev. 2010;24:2205–2218. DOI: 10.1101/gad.1972310

[70] Kim Y, Kim E, Wu Q, Guryanova O, Hitomi M, Lathia JD, Serwanski D, Sloan AE, Weil RJ, Lee J, Nishiyama A, Bao S, Hjelmeland AB, Rich JN: Platelet-derived growth factor receptors differentially inform intertumoral and intratumoral heterogeneity. Genes Dev. 2012;26:1247–1262. DOI: 10.1101/gad.193565.112

[71] Westermark B: Platelet-derived growth factor in GBM-driver or biomarker? Ups J Med Sci. 2014;119:298–305. DOI: 10.3109/03009734.2014.970304

[72] Lönn S, Rothman N, Shapiro WR, Fine HA, Selker RG, Black PM, Loeffler JS, Hutchinson AA, Inskip PD: Genetic variation in insulin-like growth factors and brain tumor risk. Neuro-Oncol. 2008;10:553–559. DOI: 10.1215/15228517-2008-026

[73] Pardo OE, Wellbrock C, Khanzada UK, Aubert M, Arozarena I, Davidson S, Bowen F, Parker PJ, Filonenko VV, Gout IT, Sebire N, Marais R, Downward J, Seckl MJ: FGF-2 protects small cell lung cancer cells from apoptosis through a complex involving PKCepsilon, B-Raf and S6K2. The EMBO Journal. 2006;25:3078–3088.

[74] Haley EM, Kim Y: The role of basic fibroblast growth factor in GBM multiforme and GBM stem cells and in their in vitro culture. Cancer Lett. 2014;346:1–5. DOI: 10.1016/j.canlet.2013.12.003

[75] Desjardins A, Friedman HS: Bevacizumab therapy for GBM: a passionate discussion. CNS Oncol. 2014;3:1–3. DOI: 10.2217/cns.13.53

[76] Wang K, Wang YY, Wang JF, Ma J, Jiang T, Dai JP: Radiologic features and expression of vascular endothelial growth factor stratify survival outcomes in patients with GBM. AJNR Am J Neuroradiol. 2016;37:629-635. DOI:10.3174/ajnr.A4567

[77] Santibanez JF, Quintanilla M, Bernabeu C: TGF-beta/ TGF-beta receptor system and its role in physiological and pathological conditions. Clin Sci (Lond). 2011;121:233–251. DOI: 10.1042/CS20110086

[78] Weiss A, Attisano L: The TGFbeta superfamily signaling pathway. Wiley Interdisciplinary Reviews. Developmental Biol. 2013;2:47–63. DOI: 10.1002/wdev.86

[79] Kaminska B, Kocyk M, Kijewska M: TGF beta signaling and its role in glioma pathogenesis. Adv Exp Med Biol. 2013;986:171–187. DOI: 10.1007/978-94-007-4719-7_9

[80] Frei K, Gramatzki D, Tritschler I, Schroeder JJ, Espinoza L, Rushing EJ, Weller M: Transforming growth factor-β pathway activity in GBM. Oncotarget. 2015;6:5963–5977.

[81] Organ SL, Tsao MS: An overview of the c-MET signaling pathway. Ther Adv Med Oncol. 2011;3:7–19. DOI: 10.1177/1758834011422556

[82] Kwak Y, Kim SI, Park CK, Paek SH, Lee ST, Park SH: C-MET overexpression and amplification in gliomas. Int J Clin Exp Pathol. 2015;8:14932–14938.

[83] Kong DS, Song SY, Kim DH, Joo KM, Yoo JS, Koh JS, Dong SM, Suh YL, Lee JI, Park K, Kim JH, Nam DH: Prognostic significance of c-Met expression in GBMs. Cancer. 2009;115:140–148. DOI: 10.1002/cncr.23972

[84] Basilico C, Arnesano A, Galluzzo M, Comoglio PM, Michieli P: A high affinity hepatocyte growth factor-binding site in the immunoglobulin-like region of Met. J Biol Chem. 2008;283:21,267–21,277. DOI: 10.1074/jbc.M800727200

[85] Xie Q, Bradley R, Kang L, Koeman J, Ascierto ML, Worschech A, De Giorgi V, Wang E, Kefene L, Su Y, Essenburg C, Kaufman DW, DeKoning T, Enter MA, O'Rourke TJ, Marincola FM, Vande Woude GF: Hepatocyte growth factor (HGF) autocrine activation

predicts sensitivity to MET inhibition in GBM. Proc Natl Acad Sci USA. 2012;109:570–575. DOI: 10.1073/pnas.1119059109

[86] Garcia-Navarrete R, Garcia E, Arrieta O, Sotelo J: Hepatocyte growth factor in cerebrospinal fluid is associated with mortality and recurrence of GBM, and could be of prognostic value. J Neurooncol. 2010;97:347–351. DOI: 10.1007/s11060-009-0037-8

[87] Watson LA, Goldberg H, Bérubé NG: Emerging roles of ATRX in cancer. Epigenomics. 2015;7:1365–13678. DOI: 10.2217/epi.15.82

[88] Liu XY, Gerges N, Korshunov A, Sabha N, Khuong-Quang DA, Fontebasso AM, Fleming A, Hadjadj D, Schwartzentruber J, Majewski J, Dong Z, Siegel P, Albrecht S, Croul S, Jones DT, Kool M, Tonjes M, Reifenberger G, Faury D, Zadeh G, Pfister S, Jabado N: Frequent ATRX mutations and loss of expression in adult diffuse astrocytic tumors carrying IDH1/IDH2 and TP53 mutations. Acta Neuropathol. 2012;124:615–625. DOI: 10.1007/s00401-012-1031-3

[89] Appin CL, Brat DJ: Molecular pathways in gliomagenesis and their relevance to neuropathologic diagnosis. Adv Anat Pathol. 2015;22:50–58. DOI: 10.1097/PAP.0000000000000048

[90] Kleinschmidt-DeMasters BK, Aisner DL, Birks DK, Foreman NK: Epithelioid GBMs show a high percentage of BRAF V600E mutation. Am J Surg Pathol. 2013;37:685–698. DOI: 10.1097/PAS.0b013e31827f9c5e

[91] Schindler G, Capper D, Meyer J, Janzarik W, Omran H, Herold-Mende C, Schmieder K, Wesseling P, Mawrin C, Hasselblatt M, Louis DN, Korshunov A, Pfister S, Hartmann C, Paulus W, Reifenberger G, von Deimling A: Analysis of BRAF V600E mutation in 1320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. Acta Neuropathol. 2011;121:397–405. DOI: 10.1007/s00401-011-0802-6

[92] Gutmann DH: BRAF-V600E mutation in pediatric and adult GBM. Neuro-Oncology. 2014;16:318–319. DOI: 10.1093/neuonc/not146

[93] Takahashi Y, Akahane T, Sawada T, Ikeda H, Tempaku A, Yamauchi S, Nishihara H, Tanaka S, Nitta K, Ide W, Hashimoto I, Kamada H: Adult classical GBM with a BRAFV600E mutation. World J Surg Oncol. 2015;13:100. DOI: 10.1186/s12957-015-0521-x

[94] Lewis PW, Elsaesser SJ, Noh KM, Stadler SC, Allis CD: Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres. Proc Natl Acad Sci USA. 2010;107:14,075–14,080. DOI: 10.1073/pnas.1008850107

[95] Schwartzentruber J, Korshunov A, Liu XY, Jones DTW, Pfaff E, Jacob K, Sturm D, Fontebasso AM, Quang DA, Tonjes M, Hovestadt V, Albrecht S, Kool M, Nantel A, Konermann C, Lindroth A, Jäger N, Rausch T, Ryzhova M, Korbel JO, Hielscher T, Hauser P, Garami M, Klekner A, Bognar L, Ebinger M, Schuhmann MU, Scheurlen W,

Pekrun A, Frühwald MC, Roggendorf W, Kramm C, Dürken M, Atkinson J, Lepage P, Montpetit A, Zakrzewska M, Zakrzewski K, Liberski PP, Dong Z, Siegel P, Kulozik AE, Zapatka M, Guha A, Malkin D, Felsberg J, Reifenberger G, von Deimling A, Ichimura K, Collins VP, Witt H, Milde T, Witt O, Zhang C, Castelo-Branco P, Lichter P, Faury D, Tabori U, Plass C, Majewski J, Pfister SM, Jabado N: Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric GBM. Nature. 2012;482:226–231. DOI: 10.1038/nature10833

[96] Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DT, Konermann C, Pfaff E, Tönjes M, Sill M, Bender S, Kool M, Zapatka M, Becker N, Zucknick M, Hielscher T, Liu XY, Fontebasso AM, Ryzhova M, Albrecht S, Jacob K, Wolter M, Ebinger M, Schuhmann MU, van Meter T, Frühwald MC, Hauch H, Pekrun A, Radlwimmer B, Niehues T, von Komorowski G, Dürken M, Kulozik AE, Madden J, Donson A, Foreman NK, Drissi R, Fouladi M, Scheurlen W, von Deimling A,Monoranu C, Roggendorf W, Herold-Mende C, Unterberg A, Kramm CM, Felsberg J, Hartmann C, Wiestler B, Wick W, Milde T, Witt O, Lindroth AM, Schwartzentruber J, Faury D, Fleming A, Zakrzewska M, Liberski PP, Zakrzewski K, Hauser P, Garami M, Klekner A, Bognar L, Morrissy S, Cavalli F, Taylor MD,van Sluis P, Koster J, Versteeg R, Volckmann R, Mikkelsen T, Aldape K, Reifenberger G, Collins VP, Majewski J, Korshunov A, Lichter P, Plass C, Jabado N, Pfister SM: Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of GBM. Cancer Cell. 2012;22:425–437. DOI: 10.1016/j.ccr.2012.08.024

[97] Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA: Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339:957–959. DOI: 10.1126/science.1229259

[98] Simon M, Hosen I, Gousias K, Rachakonda S, Heidenreich B, Gessi M, Schramm J, Hemminki K, Waha A, Kumar R: TERT promoter mutations: a novel independent prognostic factor in primary GBMs. Neuro Oncol. 2015;17:45–52. DOI: 10.1093/neuonc/nou158

[99] Veeriah S, Taylor BS, Meng S, Fang F, Yilmaz E, Vivanco I, Janakiraman M, Schultz N, Hanrahan AJ, Pao W, Ladanyi M, Sander C, Heguy A, Holland EC, Paty PB, Mischel PS, Liau L, Cloughesy TF, Mellinghoff IK, Solit DB, Chan TA: Somatic mutations of the Parkinson's disease-associated gene PARK2 in glioblastoma and other human malignancies. Nat Genet. 2010;42:77–82. DOI: 10.1038/ng.491

[100] Lin DC, Xu L, Chen Y, Yan H, Hazawa M, Doan N, Said JW, Ding LW, Liu LZ, Yang H, Yu S, Kahn M, Yin D, Koeffler HP: Genomic and Functional Analysis of the E3 Ligase PARK2 in Glioma. Cancer Res. 2015;75:1815–1827. DOI: 10.1158/0008-5472.CAN-14-1433

[101] Von Deimling A, Korshunov A, Hartmann C: The next generation of glioma biomarkers: MGMT methylation, BRAF fusions and IDH1 mutations. Brain Pathol. 2011;21:74–87. DOI: 10.1111/j.1750-3639.2010.00454.x

[102] Glas M, Happold C, Rieger J, Wiewrodt D, Bähr O, Steinbach JP, Wick W, Kortmann RD, Reifenberger G, Weller M, Herrlinger U: Long-term survival of GBM patients treated with radiotherapy and lomustine plus temozolomide. J Clin Oncol. 2009;27:1257–1261. DOI: 10.1200/JCO.2008.19.2195

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