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TOPIC REVIEW

Recent advances in the molecular understanding of glioblastoma

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Abstract Glioblastoma is the most common and most aggressive primary brain tumor. Despite maximum treatment, patients only have a median survival time of 15 months, because of the tumor's resistance to current therapeutic approaches. Thus far, methylation of the O^6-methylguanine-DNA methyltransferase (MGMT) promoter has been the only confirmed molecular predictive factor in glioblastoma. Novel "genome-wide" techniques have identified additional important molecular alterations as mutations in isocitrate dehydrogenase 1 (*IDH1*) and its prognostic importance. This review summarizes findings and techniques of genetic, epigenetic, transcriptional, and proteomic studies of glioblastoma. It provides the clinician with an up-to-date overview of current identified molecular alterations that should ultimately lead to new therapeutic targets and more individualized treatment approaches in glioblastoma.

Keywords Glioblastoma · Molecular · (Epi)genetic · Transcriptional · Proteomic

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Introduction

Glioblastoma, or astrocytoma WHO grade IV, is the most fatal primary brain cancer found in humans. Most glioblastomas manifest rapidly de novo, without recognizable precursor lesions. These primary glioblastomas present in elderly patients with a brief clinical history and are characterized by rapid progression and short survival time. A small group of young patients has a history of epilepsy caused by low-grade gliomas which, within years, progress to secondary glioblastoma. A secondary glioblastoma occurs in ~5% of glioblastoma patients, and can only be diagnosed with clinical (neuroimaging) or histological evidence of its evolution from a less malignant glioma [1].

The standard treatment for newly diagnosed glioblastoma patients is gross total removal, if possible, followed by the combination of the alkylating cytostatic drug temozolomide (TMZ) and RT [2, 3]. Median overall survival is 15 months only [3], although for a rare group of long-term survivors (2–5%) survival time exceeds 3 years [4, 5]. Differences between patients and their performance status lead to variation in survival, which can be calculated for individual patients by means of nomograms [6]. A better prognosis is associated with younger age, better performance status, and more extensive surgical resection followed by TMZ and RT [6]. In contrast with many other malignancies, however, there have only been small improvements in the glioblastoma patient's prognosis over recent decades. Nevertheless, understanding of the molecular alterations in signaling pathways and the consequent pathology in glioblastoma has greatly increased in recent years and is beginning to match that of other types of cancer.

This review provides an overview of the molecular alterations in glioblastoma (Fig. 1) [7–9]. They are

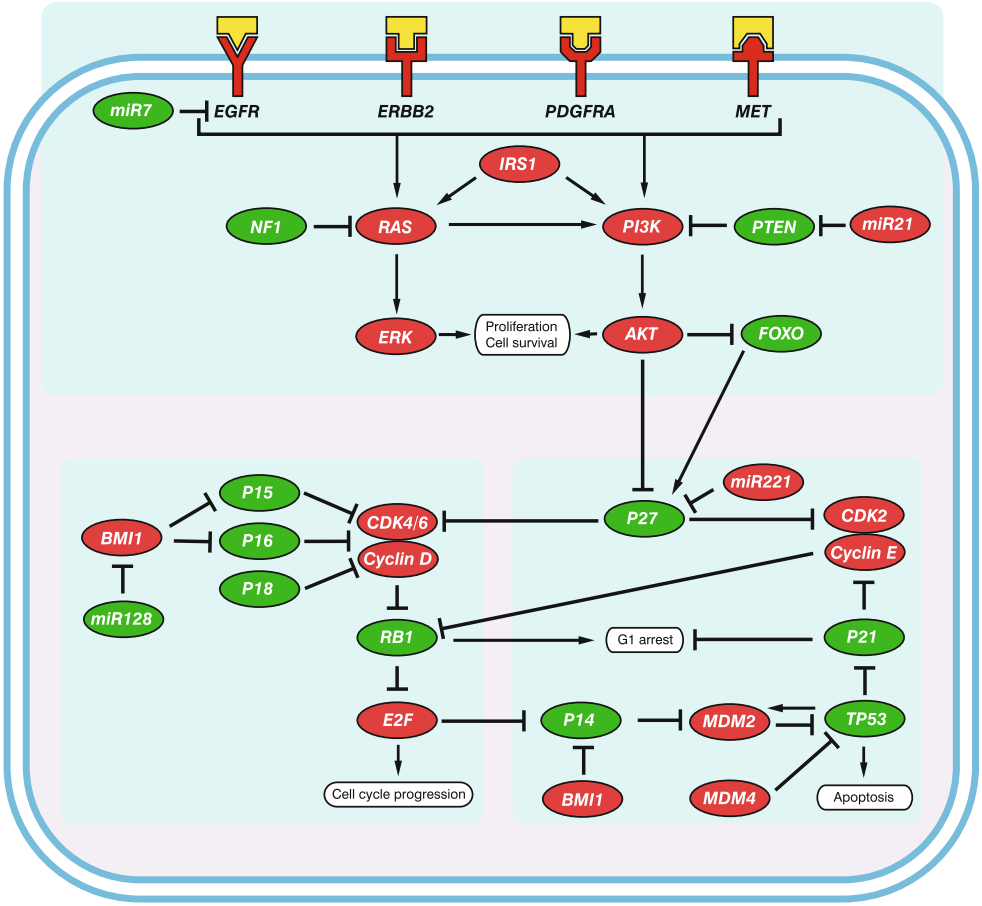

Fig. 1 Simplified representation and integration of three commonly altered pathways involved in glioblastoma. *Upper panel*, the growth factor receptor/PI3K/AKT pathway. The *lower panels* depict the RB pathway (*left*) and the P53 pathway (*right*). Proteins that potentially act as tumor suppressors are indicated in *green* whereas oncoproteins are indicated in *red*. The growth factors binding to the receptors have been depicted in *yellow*

grouped according to the different mechanisms that underlie transformation to the neoplastic phenotype, starting from (epi)genetic, via transcriptional, to proteomic studies of glioblastoma. The important molecular alterations, which have been identified by novel "genome-wide" techniques, are discussed in relation to gliomagenesis and glioma progression and in relation to clinical subgroups and prognosis. Finally, we discuss the application of these new insights in the light of future prospects for experimental and clinical practice in neuro-oncology.

Genomic and genetic variants

Genomic instability

Genomic instability is one of the enabling characteristics of cancer [10]. It can be broadly differentiated into chromosome instability (CIN) and microsatellite instability (MIN or MSI). Cytogenetic studies of glioblastoma have shown that most tumors are near-diploid, and that numerical and structural chromosomal abnormalities are common [11]. MSI is rarely observed for non-inherited newly diagnosed glioblastomas, because of inactivation of mismatch repair (MMR) genes [12]. However, in recurrent glioblastomas after TMZ treatment, inactivating mutations have been observed in *MSH6*, one of the MMR genes. *MSH6* mutations have not been associated with detectable MSI as manifested by changes in the length of microsatellite sequences, but with a hypermutator phenotype [7, 9, 13]. As genetic alterations and genomic instability are closely linked with each other, it is an interesting finding that in glioblastoma, tumors from short-term survivors have more genetic alterations than long-term survivors' tumors [5].

Chromosomal alterations

Techniques

Evolving techniques have identified increasingly more detailed chromosomal alterations.

Karyograms [11], fluorescent in situ hybridization (FISH) analyses [14], and comparative genomic hybridization (CGH) [15, 16] have preceded whole-genome single nucleotide polymorphism (SNP)-based arrays. Whereas karyograms are able to reveal only gross chromosomal changes, SNP-based arrays have the ability to detect copy number alterations (CNAs), varying from complete

chromosomal changes to small intragenic deletions. In addition, it is possible to distinguish signals from individual alleles and therefore reveal copy-number-neutral (CNN) loss of heterozygosity (LOH). Here, a chromosome segment is lost, whereas the corresponding homologous region is duplicated, resulting in a neutral copy number. For example, 17p, which contains *TP53*, is a significant region of CNN LOH in glioblastoma [7, 8].

Among chromosomal alterations, amplifications and deletions can be distinguished. Of these, the most common in glioblastoma will be discussed here. Reports of incidental translocations are rare in glioblastoma [17]; consequently, translocations may not be important in the development of glioblastoma and will not be discussed further.

Amplifications

Amplification of the epidermal growth factor receptor (*EGFR*) gene is a characteristic finding in primary glioblastoma (Table 1) [5, 8, 16, 18]. Focal (restricted to a few Mb) and broader (from several Mbs to whole chromosomes) CNAs that include the *EGFR* gene may have different molecular consequences [16]. Focal amplification of *EGFR* correlates with EGFR overexpression or mutations and deletions in the *EGFR* gene, and subsequent activation of the PI3K/AKT pathway [16, 19]. Upregulated PI3K/AKT signaling has been associated with a poor prognosis [20, 21]. Amplification of the complete chromosome 7, containing *EGFR*, *MET* [7], and its ligand *HGF*, has been found to correlate with activation of the MET axis [16]. Furthermore, *EGFR* amplification is reported to appear as double minutes (small fragments of extrachromosomal DNA), and extra copies of *EGFR* have also been found inserted into different loci on chromosome 7 [22]. Remarkably, gain of chromosome 7 and amplification of *EGFR* have been found more frequently in short-term survivors [4, 5], however *EGFR* alterations are not of prognostic importance in glioblastoma [4, 18, 23].

Amplification of 12q13-15, where the oncogenes *CDK4* and *MDM2* are located, results in the disruption of both the RB and P53 pathways [7, 8, 16, 24]. The genes encoding the receptor tyrosine kinases *KIT*, *KDR*, and *PDGFRA*, adjacently located on chromosome 4q12, are frequently found to be (co)amplified [25]. Other amplified regions containing oncogenes, for example *AKT3* [7, 26] and *CCND2* [7, 16], are listed in Table 1.

Deletions

LOH of chromosome 10q is the most common genomic alteration found in both primary and secondary glioblastomas [18, 24] (Table 1) and is associated with poor survival [5, 18]. Different regions are frequently lost at chromosome 10, including the regions containing *PTEN*, *MGMT* [1, 18], and *ANXA7*, an EGFR inhibitor [27]. Another frequently deleted inhibitor of EGFR signaling is *NFKBIA,* which is located on chromosome 14; this deletion is associated with poor survival [28]. Furthermore, loss of chromosome 9p, which contains a variety of tumor-suppressor genes, including *CDKN2A, CDKN2B*, and *PTPRD,* is frequently seen [8, 18, 29], especially in short-term survivors [4, 5]. *CDKN2A* and *CDKN2B* encode three important cell cycle proteins, $p14^{ARF}$ and $p16^{INK4A}$, and $p15^{INK4B}$ [5, 8, 15, 16, 18], which are involved in the RB and P53 pathways. Deletion of *CDKN2A* and *CDKN2B* is often accompanied by deletion of *CDKN2C* on chromosome 1p32, which encodes another cell cycle protein $p18^{INK4C}$ [15]. LOH of chromosome 1p is found in both primary and secondary glioblastomas [30]. Longstanding speculation about the potentially located tumor suppressor gene at 1p has recently been advanced by identification of the suggested candidate genes *CIC* and *FUPB1* [31]. Co-deletion of 1p and 19q is frequently seen in oligodendrogliomas and is, in those, associated with prolonged survival [4] and translocations [32]. Although this co-deletion has been observed in glioblastomas, no similar association has been identified. Isolated LOH 19q, however, is frequently observed in secondary glioblastoma [5, 30] and may be a marker of longer survival [5].

Somatic mutations

Techniques

In addition to amplifications and deletions, genes implicated in glioblastoma can be affected by somatic mutations. Mutation analysis has identified mutations activating oncogenes and others inactivating tumor-suppressor genes in glioblastoma [7, 9, 33]. The recommended method used to be direct or Sanger sequencing after amplification of the suspected locus by means of polymerase chain reaction (PCR). Nowadays, improved sequencing techniques are being developed and rapidly applied to facilitate genome-wide mutation analysis [34].

Mutations frequently found in glioblastoma

Mutations in "common" cancer genes, for example *TP53* and *PTEN*, are very frequent in glioblastomas, but are not of prognostic importance (Table 2) [4, 7, 9, 18, 23, 33]. Furthermore, glioblastoma-specific mutations are seen; the *EGFRvIII* mutant lacks 267 amino acids in the extracellular part, resulting in a constitutively activated receptor that no longer requires its ligand EGF to signal downstream [35]. *EGFR* point mutations have also been identified in

Table 1 Frequently identified copy number alterations in glioblastoma

Chromosome	Cytoband	Alteration	Frequency (%)	Gene symbol	Gene name	Function of encoded protein	Refs.
1	1p32	Deletion	3–16	CDKN2C	Cyclin-dependent kinase inhibitor 2C	Regulator of cell cycle	[7, 24]
1	1p36	Deletion	14–40	?[a]			[8, 26, 30]
1	1q32	Amplification	4–15	MDM4	Mdm4 p53 binding protein homolog	Apoptosis	[7, 8, 16, 24]
1	1q44	Amplification	2–11	AKT3	V-akt murine thymoma viral oncogene homolog 3	Regulator of cell signaling, involved in cell proliferation and survival	[7, 24, 26]
2	2q22	Deletion	7	LRP1B	LRP1B low density lipoprotein receptor-related protein 1B	Regulator of cell signaling, involved in cell proliferation and survival	[8]
3	3q26	Amplification	0–16	PIK3CA	Phosphoinositide-3-kinase, catalytic, alpha polypeptide	Regulator of cell signaling, involved in cell proliferation and survival	[7, 9, 16]
4	4q12	Amplification	15	KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	Regulator of cell signaling, involved in cell proliferation and survival	[25]
4	4q12	Amplification	15	KDR	Kinase insert domain receptor	Angiogenesis, vasculogenesis and endothelial cell growth	[25]
4	4q12	Amplification	2–18	PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide	Regulator of cell signaling, involved in cell proliferation and survival	[7, 8, 16, 24, 25]
6	6q26-27	Deletion	25	PARK2	Parkinson protein 2	Regulator in targeting proteins for proteasomal degradation	[8]
7	7p11	Amplification	23–66	EGFR	Epidermal growth factor receptor	Regulator of cell signaling, involved in cell proliferation and survival	[7–9, 16, 18, 24]
7	7q21	Amplification	1	CDK6	Cyclin-dependent kinase 6	Regulator of cell cycle	[7]
7	7q31	Amplification	3–19	MET	Met proto-oncogene	Regulator of cell signaling, involved in cell proliferation and survival	[7, 16, 24]
9	9p21	Deletion	26–66	CDKN2A	Cyclin-dependent kinase inhibitor 2A	Regulator of cell cycle	[7–9, 18, 24]
9	9p21	Deletion	31–66	CDKN2B	Cyclin-dependent kinase inhibitor 2B	Regulator of cell cycle	[7, 8, 24]
9	9p23	Deletion	14–46	PTPRD	Protein tyrosine phosphatase, receptor type, D	Regulator of cell signaling, involved in cell proliferation and survival	[24]
10	10q23-24	Deletion	5–70	PTEN	Phosphatase and tensin homolog	Regulator of cell signaling, involved in cell proliferation and survival	[7–9, 16, 18, 24]
12	12p13	Amplification	2–14	CCND2	Cyclin D2	Regulator of cell cycle	[7, 16]
12	12q14	Amplification	7–24	CDK4	Cyclin-dependent kinase 4	Regulator of cell cycle	[7–9, 16, 24]
12	12q14-15	Amplification	7–22	MDM2	Mdm2 p53 binding protein homolog	Apoptosis	[7, 8, 16, 24]
13	13q14	Deletion	3–47	RB1	Retinoblastoma 1	Regulator of cell cycle	[7–9, 16, 30]
17	17p13	Deletion	1–22	TP53	Tumor protein p53	Apoptosis	[7–9, 16]
17	17q11	Deletion	0–11	NF1	Neurofibromin 1	Regulator of cell signaling, involved in cell proliferation and survival	[7, 9]
19	19q	Deletion	11–35	?			[16, 24, 30]
22	22q12.3	Deletion	53	TIMP3	TIMP metallopeptidase inhibitor 3	Extracellular matrix	[77]

A deletion can indicate either a CNN-LOH, an LOH, or a homozygous deletion; a ? indicates that the gene of interest has not yet been identified

[a] Genes within the region: CAMTA1, PER3, UTS2, TNFSF9, VAMP3, PARK7, MIG6, RERE, GPR157, H6PD [8]

Table 2 Genes frequently found to be mutated in glioblastoma

Gene symbol	Gene name	Function of encoded protein	Point mutation (%)	Refs.
EGFR	Epidermal growth factor receptor	Regulator of cell signaling, involved in cell proliferation and survival	14–15	[7, 9, 36]
ERBB2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2	Regulator of cell signaling, involved in cell proliferation and survival	0–7	[7, 9]
IDH1	Isocitrate dehydrogenase 1 (NADP+)	NADPH production	12–20	[9, 39–42, 44]
NF1	Neurofibromin 1	Regulator of cell signaling, involved in cell proliferation and survival	15–17	[7, 9]
PIK3CA	Phosphoinositide-3-kinase, catalytic, alpha polypeptide	Regulator of cell signaling, involved in cell proliferation and survival	7–10	[7, 9]
PIK3R1	Phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	Regulator of cell signaling, involved in cell proliferation and survival	7–8	[7, 9]
PTEN	Phosphatase and tensin homolog	Regulator of cell signaling, involved in cell proliferation and survival	24–37	[7, 9, 18]
PTPRD	Protein tyrosine phosphatase, receptor type, D	Regulator of cell signaling, involved in cell proliferation and survival	0–6	[9]
RB1	Retinoblastoma 1	Regulator of cell cycle	8–13	[7, 9]
TP53	Tumor protein p53	Apoptosis	31–38	[7, 9, 18]

glioblastoma, in the extracellular domain, whereas they are predominantly found in the kinase domain in other tumor types, for example lung cancer [36]. Two extensive mutational studies have provided an overview of the most common mutations affecting glioblastoma (Table 2) [7, 9]. Although mutations in "common" cancer genes, for example *BRAF* and the *RAS* genes, have rarely been observed in gliomas (<5%) [37], inactivating mutations and deletions have been identified in their inhibitory tumor suppressor gene *NF1* [7]. Mutations in *PIK3CA* and *PIK3R1*, coding, respectively, for the PI3K catalytic subunit p110α and regulatory subunit P85α, have been described [7, 9].

The incidence of mutation in glioblastoma is lower than in other solid tumors [38], with the exception of the hypermutator phenotype [13], which, as described above, is found in recurrent glioblastomas after treatment with alkylating agents. This may be caused by *MGMT* methylation or mutational inactivation of DNA-repair enzymes, for example *MSH6* [7, 9, 13].

IDH1 mutations

An interesting gene found to contain mutations in glioblastoma is *IDH1*, which encodes isocitrate dehydrogenase 1 and is involved in energy metabolism [9]. *IDH1* mutations have been predominantly identified in secondary glioblastomas and low-grade gliomas, with mutations in more than 70% of cases [9, 39–43]; they are found only sporadically in primary glioblastomas [9, 41–44]. Because patients with *IDH1* mutated primary glioblastomas are generally younger and have longer median survival and wild-type *EGFR*, which are characteristics of secondary glioblastomas, it is hypothesized that these are in fact secondary glioblastomas for which no histological evidence of evolution from a less malignant glioma is found. Therefore, *IDH1* could be used to differentiate primary from secondary glioblastomas [41]. In different glioblastoma studies *IDH1* mutations have been found to be an independent positive prognostic marker [9, 40, 44, 45]. *IDH1* mutations have been shown to inactivate the enzyme with subsequent HIF-1a induction [42, 44, 46]. In addition, the mutations result in gain of function to catalyze α-ketoglutarate (α-KG) to 2-hydroxyglutarate (2-HG) [47]. Furthermore, 2-HG inhibits histone demethylases and TET 5-methylcytosine hydroxylases. These α-KG dependent dioxygenases are thought to be involved in epigenetic control. This suggests that mutations in *IDH1* change the expression of a potentially large number of genes [48]. Given that mutations in *IDH1* are an early event in gliomagenesis (Fig. 2) [49], this may implicate widespread alteration of epigenetic control as the key mechanism in gliomagenesis in *IDH1* mutated tumors. Furthermore, it might explain the extensive and fundamental differences between mutated and wildtype *IDH1* glioblastoma.

Polymorphisms

Family members of glioma patients are more susceptible to glioma and other cancer types [50], suggesting a genetic origin. The most common type of genetic variation is formed by single nucleotide polymorphisms (SNPs). A SNP is a single base-pair alteration at a specific locus. They can be identified by PCR for single loci or use of

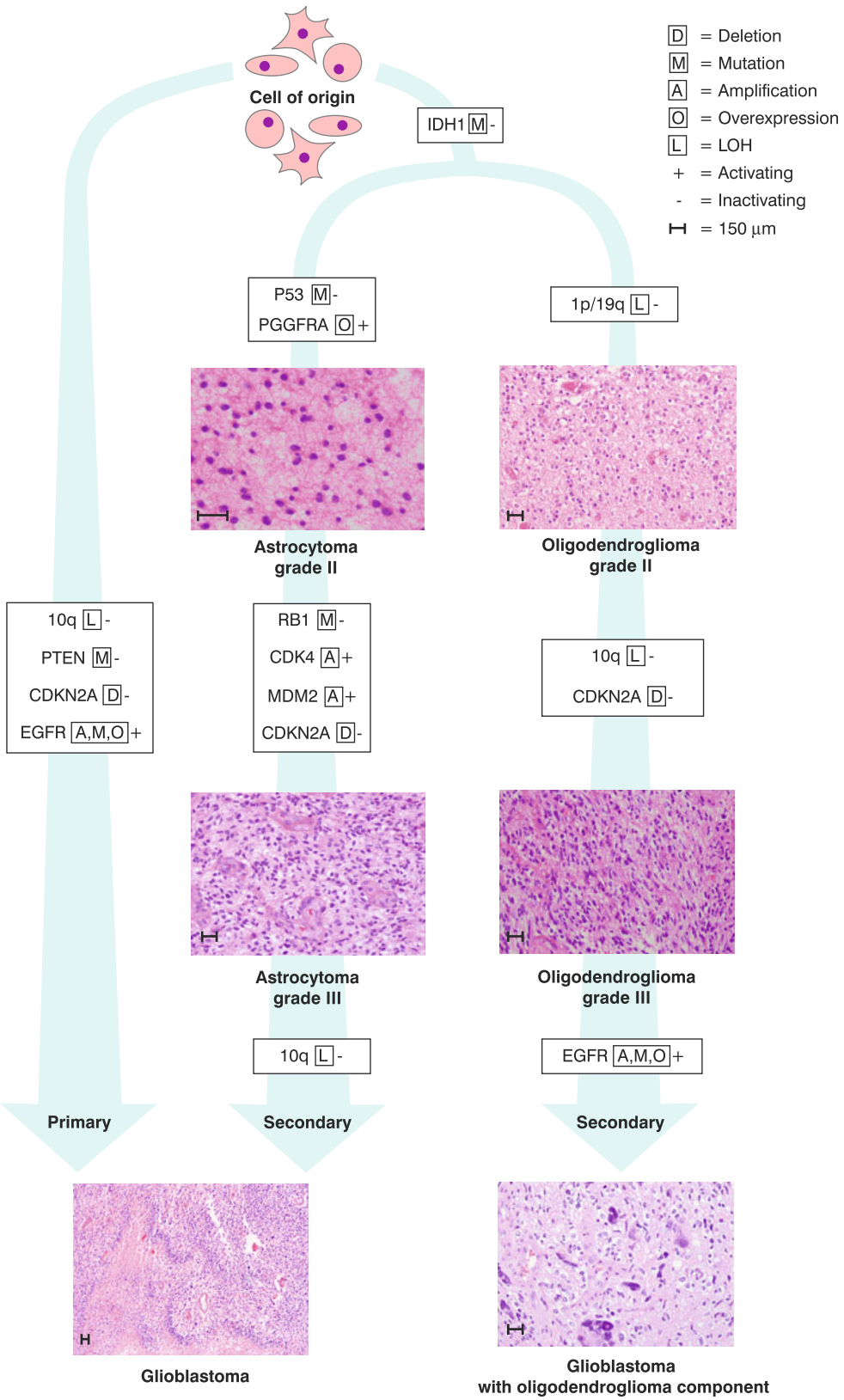

Fig. 2 Genetic pathways toward primary and secondary glioblastoma

SNP-based arrays for whole genome alterations. SNPs have been linked to susceptibility to glioblastomas. In particular, allergies and asthma's inverse association with glioblastoma have been observed in different studies and have been linked with polymorphisms in HLA and interleukins. This may suggest that immune factors play a role

in gliomagenesis [51]. SNP309 in *MDM2* has been associated with an increased risk of various types of cancer, but has not been associated as a risk or prognostic factor in respect of glioblastoma in large studies [52]. SNPs in *CDKN2B*, *TERT*, and *RTEL1* have been described in independent studies as susceptibility loci for high-grade glioma [53, 54]. In a follow-up study, SNPs in DNA double-strand break repair enzymes, for example *RTEL1*, have been found to correlate with glioblastoma survival [55]. Various other SNPs have been correlated with glioblastoma survival and age of onset [55], however, these studies' findings have not yet been confirmed.

Gene expression profiling

Techniques and results

Overexpression or underexpression of genes in glioblastoma compared with that in a normal brain or in low-grade gliomas may be an indication of genes that are involved in gliomagenesis (Table 3). Most of the 20,000–25,000 genes encoded by the human genome are known [56], and these have been applied to chips used for micro-arrays. Differences in expression of "unknown" genes can be studied by serial analysis of gene expression (SAGE), by use of small expression tags [57]. Large-scale expression studies are usually validated by reverse transcription (RT)-PCR for individual genes.

A high level of expression of insulin-like growth factor binding proteins, for example IGFBP-2/3 [58], angiogenesic factors, for example vascular endothelial growth factor A (VEGFA) [59], and mesenchymal markers, for example YKL-40/CHI3L1, are frequently seen in glioblastoma (Table 3) and have been associated with poor prognosis [60–62]. In contrast, NOTCH signaling genes, for example DLL3, are indicative of better survival [63]. Furthermore, WEE1, a kinase that regulates the G_2 checkpoint in glioblastoma cells, is commonly overexpressed in glioblastoma and higher expression has been shown to correlate with worse patient survival [64].

Gene expression profiling studies outperform histology for grading and prognosis

Low-grade astrocytomas have rather specific and consistent expression profiles, whereas for primary glioblastomas there is much larger variation between tumors. Furthermore, secondary glioblastomas have distinct expression profiles and features of the other two types [65]. Expression profiling of different types and grades of glioma has been found to outperform histopathologic grading for prognosis [20, 66–68]. To improve classification of patients with glioblastoma, a gene dosage expression incorporated model based on seven genes (*POLD2*, *CYCS*, *MYC*, *AKR1C3*, *YME1L1*, *ANXA7*, and *PDCD4*) has been generated. This model can be used to categorize patients in risk groups with different prognosis; a high-risk group in which $\geq$5 of 7 genes are altered, a moderate-risk (3–4 genes), or a low-risk group ($\leq$2 genes). In this study, *MGMT* methylation and *IDH1* mutational status were not incorporated [69]. A newer predictive model based on expression of four genes (*CHAF1B, PDLIM4, EDNRB*, and *HJURP*) has been generated, and is independent of *MGMT* methylation and

Table 3 Genes frequently found to be overexpressed in glioblastoma compared with either normal brain tissue or low-grade gliomas

Gene symbol	Gene name	Function of encoded protein	Refs.
CD44	CD44 molecule	Cell-cell interactions, cell adhesion and migration	[20, 62]
DLL3	Delta-like 3	Notch signaling	[20, 62]
EGFR	Epidermal growth factor receptor	Regulator of cell signaling, involved in cell proliferation and survival	[62]
FABP7	Fatty acid binding protein 7	Fatty acid uptake, transport, and metabolism	[62]
IGFBP2	Insulin-like growth factor binding protein 2	Regulation of cell growth	[58–60, 62]
IGFBP3	Insulin-like growth factor binding protein 3	Regulation of cell growth	[58]
MMP9	Matrix metallopeptidase 9	Extracellular matrix	[62]
SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)	Extracellular matrix	[62]
TNC	Tenascin C	Cell adhesion	[60, 62]
VEGFA	Vascular endothelial growth factor A	Angiogenesis, vasculogenesis, and endothelial cell growth	[20, 59, 60, 62]
CHI3L1	Chitinase 3-like 1(YKL-40)	Extracellular matrix	[20, 60, 62]
VIM	Vimentin	Cytoskeletal element	[20]

IDH1 mutational status. Here, high expression of EDNRB correlates with longer survival whereas the other genes are correlated with higher risk of death. On the basis of the expression of these 4 genes, low-risk and high-risk groups were formed. Interestingly, survival was similar for patients in the low-risk group with wildtype *IDH1* and patients in the high-risk group with mutated *IDH1* [70].

Expression classification and prognosis according to TCGA studies

Studies by The Cancer Genome Atlas (TCGA) have incorporated genomic alterations within expression analyses. Distinct molecular subclasses in high-grade glioma have been identified, delineating a pattern of disease progression that resembles stages in neurogenesis, and have been used to classify glioblastomas into proneural, neural, classic, and mesenchymal subtypes [20, 63, 71]. Proneural glioblastomas are characterized by *IDH1* mutations, and *TP53* and *PDGFRA* alterations, and correlate with a better prognosis and younger age. Classic glioblastomas are differentiated on the basis of high-level amplification of *EGFR,* monosomy of chromosome 10, and deletion of *CDKN2A*. Neural glioblastomas are typified by expression of neuron markers, and resemble normal brain most. Mesenchymal glioblastomas are known for *NF1* deletion or mutation and expression of YKL-40/CHI3L1 and MET [20, 71]. Different subtypes of glioblastoma have been shown to behave differently in response to treatment; Classic and mesenchymal subtypes have a survival advantage after TMZ and RT, whereas the proneural subtype of glioblastomas, with relative good prognostic, does not [71]. Stratified clinical trials in which patient inclusion is based on the genetic alterations that have been identified in their tumor samples are necessary to further increase our understanding of the clinical possibilities of these subgroups.

Epigenetics

Epigenetic silencing mechanisms

Epigenetic silencing of tumor suppressor genes is a common phenomenon of genomic instability in cancer [10]. Epigenetics are inherited characteristics of gene expression, not related to nucleotide sequences. Examples are promoter hypermethylation, histone deacetylation, histone methylation, other histone modifications which can alter chromatin structure (in)directly, and RNA-silencing mechanisms such as RNA interference and microRNA (miRNA or miR) regulation of gene expression [72]. In contrast with the global DNA hypomethylation found in glioblastoma and other tumors [73], tumor suppressor genes are commonly found to be hypermethylated and, hence, silenced [72]. DNA methylation, histone deacetylation, and miRs are best studied in glioblastoma and are discussed next.

Methylation and histone deacetylation

In glioblastoma, similar to other cancers, global DNA hypomethylation is often seen with hypermethylation of CpG islands in promoter regions. Tumor-suppressor genes frequently found to be silenced by hypermethylation in glioblastoma include *CDKN2A*, *CDKN2B*, *RB1, PTEN*, and *TP53*. (reviewed elsewhere [74, 75]). Differences in various genes' promoter methylation have been found between primary and secondary glioblastomas (Table 4) [76–78], long and short-term glioblastoma survivors [75, 79], primary and recurrent tumors, and time to tumor progression [80].

MGMT methylation

Particularly important in glioblastoma is the methylation status of *MGMT*, which is a predictive factor for therapy response and hence survival of glioblastoma patients treated with TMZ and RT [2, 23, 81]. *MGMT* methylation has been observed in 40–57% of glioblastomas; however, specific subgroups have a higher frequency. *MGMT* methylation has been found to be more frequent in secondary glioblastomas [82], in females [83], and in long-term survivors (LTS) [4], whereas it is rare (5%) in recurrent glioblastomas [84]. Conflicting results have been reported regarding the methylation status of *MGMT* as a positive prognostic marker [74, 75, 83]. TMZ and other alkylating agents modify the O^6-position in guanines thereby forming critical DNA lesions that progress to lethal DNA cross-links which prohibit cell replication. The DNA repair enzyme MGMT is able to remove alkyl groups, thus introducing resistance to TMZ treatment. However, when the promoter of *MGMT* is methylated, *MGMT* is not transcribed and therefore cannot repair DNA damage caused by TMZ, making TMZ more efficient. The best means of assessment of the *MGMT* methylation status has been debated; the most widely recommended method is methylation-specific PCR (MSP) [85]. Recently, the methylation status of the *FNDC3B, TBX3, DGKI*, and *FSD1* promoters was identified to be important in patients with *MGMT*-methylated tumors who did not respond to TMZ and RT treatment [79]. *MGMT* methylation is also associated with pseudo-progression after concomitant radiochemotherapy for newly diagnosed glioblastoma patients [86]. Furthermore, the pattern of recurrence, including time to recurrence and location of the recurrent tumor, seems to be correlated with the *MGMT* methylation status of the primary tumor [87].

Table 4 Molecular differences between primary and secondary glioblastoma

Event	Gene symbol	Gene name	Function of encoded protein	Overall frequency in glioblastoma (%)	Frequency in primary glioblastoma (%)	Frequency in secondary glioblastoma (%)	Refs.
Amplification	EGFR	Epidermal growth factor receptor	Regulator of cell signaling, involved in cell proliferation and survival	34	36	8	[18]
Deletion	CDKN2A-P14^{ARF}	Cyclin-dependent kinase inhibitor 2A	Regulator of cell cycle	44	44	44	[18]
	CDKN2A-P16^{INK4A}	Cyclin-dependent kinase inhibitor 2A	Regulator of cell cycle	26–31	31–32	13–19	[18]
LOH	10q (including PTEN)	Phosphatase and tensin homolog	Regulator of cell signaling, involved in cell proliferation and survival	69	70	63	[30]
	13q (including RB1)	Retinoblastoma 1	Regulator of cell cycle	23	12	38	[30]
	22q (including TIMP3)	TIMP metallopeptidase inhibitor 3	Involved in degradation of the extracellular matrix	53	41	82	[77]
	19q	?		27	54	6	[30]
Methylation	MGMT	O^6-methylguanine-DNA methyltransferase	DNA repair	44	43	73	[83]
	CDKN2A-P14^{ARF}	Cyclin-dependent kinase inhibitor 2A	Regulator of cell cycle	14	6	31	[18]
	CDKN2A-P16^{INK4A}	Cyclin-dependent kinase inhibitor 2A	Regulator of cell cycle	8	3	19	[18]
	NDRG2	N-myc downstream-regulated gene 2	May have a role in neurite outgrowth	46	62	0	[83]
	PTEN	Phosphatase and tensin homolog	Regulator of cell signaling, involved in cell proliferation and survival	32	9	82	[78]
	RB1	Retinoblastoma 1	Regulator of cell cycle	25	14	43	[76]
	TIMP3	TIMP metallopeptidase inhibitor 3	Involved in degradation of the extracellular matrix	41	28	71	[77]
Mutation	IDH1	Isocitrate dehydrogenase 1 (NADP+)	NADPH production	12–20	4–12	73-88	[9, 39, 41, 42, 44]
	PTEN	Phosphatase and tensin homolog	Regulator of cell signaling, involved in cell proliferation and survival	24–37	25–40	4	[18, 33]
	TP53	Tumor protein p53	Apoptosis	31–38	28–29	65	[18, 33]

A ? indicates that the gene of interest has not yet been identified

Hypermethylation phenotype

A subset of glioblastoma tumors has been found to contain a hypermethylation phenotype at a large number of CpG islands; this has been named the glioma-CpG island methylator phenotype (G-CIMP) by the TCGA. These G-CIMP tumors cluster into the aforementioned proneural subgroup, are strongly associated with *IDH1* mutations, and generally affect younger patients with improved prognosis [88]. Furthermore, inhibition of histone demethylases and TET 5-methylcytosine hydroxylases by mutated *IDH1* potentially implies the methylation of an even greater number of genes in this subgroup [48].

MicroRNAs

miRs are short non-coding RNAs, consisting of approximately 22 nucleotides, which regulate gene expression. miRs usually inhibit target genes' expression, either by inhibiting translation or by triggering the cleavage of the target mRNA. Over 700 miRs have been described in humans [89]. By use of the same methods previously described for gene expression, differences in miR expression have been examined. Compared with normal brain tissue a variety of differentially expressed miRs have been found (Table 5) [90–101].

OncomiRs, tumor suppressor miRs, and therapeutic implications

Frequently up-regulated miRs are called oncomiRs. Of these, miR-26a is found to target PTEN in glioblastomas [102]. Furthermore, miR-26 cooperates with oncogenes *CDK4* and *CENTG1,* forming an oncomiR/oncogene cluster, targeting the RB, PI3K/AKT, and JNK pathways and increasing aggressiveness in glioblastoma [95]. miR-221 and miR-222 are thought to target cell cyclin-dependent kinase inhibitors p27 and p57 by targeting the pro-apoptotic PUMA [103]. In contrast with these oncomiRs, frequently down-regulated miRs in glioblastoma are considered tumor-suppressor miRs. Of these, miR-7 independently inhibits both the EGFR and AKT pathways [98]. miR-34a suppresses glioblastoma growth by targeting c-Met and Notch [99]. miR-124 and miR-137 target CDK6, which is important in the G1/S-phase transition [97]. miR-128 targets BMI1, which has been shown to promote stem cell renewal [94]. Downregulation of miR-181 is found in responders to temozolomide [100]. The delivery of underexpressed tumor-suppressor miRs may be an appealing approach for therapy. In contrast, overexpressed oncogenic miRNas may be targeted by antagomirs, because overexpression of the oncomiRs miR-26a, miR-196, and miR-451 has been correlated with poorer survival [93]. A recent review has provided an up-to-date overview on miRs and their inhibitors for glioblastoma treatment and readers should refer to this for more information [104].

Proteomics

Proteomic studies involve research on the final structure, function, and activity of proteins. Therefore, post-translational modifications on the transcript are included in the results. Thus far, only a limited number of proteomic studies have been performed on glioblastomas and there are still conceptual and technical limitations to overcome [105]. In general, samples are run on 2D gels, which show protein patterns on the basis of size and charge. Proteins identified in tumor samples but not in normal tissue samples are subsequently analyzed by mass spectrometry with matrix-assisted laser desorption/ionization (MALDI) [106]. Thus far, glioma subtypes have been distinguished on the basis of different protein patterns as primary and secondary glioblastomas [107, 108]. Furthermore, on the basis of proteome analysis, survival has been predicted in respect of glioma subtypes [107]. Additionally, proteins' phosphorylation status is a tool with which to identify activated proteins. Consequently, activated receptor tyrosine kinases [109, 110] and the downstream signaling pathways of EGFRvIII have been identified in glioblastomas [111].

Other molecular aspects of glioblastomas

Molecular differences between primary and secondary glioblastomas

Primary and secondary glioblastoma subtypes are histopathologically indistinguishable, but differences can be demonstrated by molecular markers at the epigenetic [77], genetic [1, 18, 24], expression [65], and proteomic [108] levels (Fig. 2; Table 4). Primary glioblastomas have a greater prevalence of EGFR alterations, *MDM2* duplications, *PTEN* mutations, and homozygous deletions of *CDKN2A* [1, 18]. *MET* amplification [24], overexpression of PDGFRA, and mutations in *IDH1* and *TP53* are more prevalent in secondary glioblastomas [1, 9, 18, 33, 39, 41, 43].

The sequential order of molecular alterations

Molecular alterations causing glioblastoma are thought to occur in a sequential order, implicating different stages of gliomagenesis (Fig. 2). For example, *IDH1*-inactivating mutations seem to be an early event in gliomagenesis [43]. In contrast, *PTEN* mutations and LOH 10q are thought to be important in glioma progression, but not initiation [18].

Table 5 Frequently identified microRNA expression alterations in glioblastoma

miRNA	Alteration of expression	Function of encoded protein	Targets	Refs.
miR-7	Decreased	Increases apoptosis, decreases invasion	EGFR	[92, 97, 98]
miR-15	Increased	Regulator of cell-cycle progression	CCNE1	[93]
miR-21	Increased	Oncomir, antiapoptosis	RECK, PDCD4, PTEN	[92, 93, 97]
miR-26	Increased	Induces tumor growth, part of oncomir/oncogene cluster with CDK4 and CENTG1	PTEN and PI3K/Akt pathway	[102]
miR-34	Decreased	Inhibitor of proliferation, survival, migration, and invasion	TP53, c-Met, NOTCH1/2	[99]
miR-124	Decreased	Inhibitor of proliferation, cell differentiation	CDK6, PTBP, SCP1	[97]
miR-125	Increased	Inductor of proliferation and inhibitor of apoptosis	ERBB2, ERBB3, TP53	[92]
miR-128	Decreased	Inhibitor of proliferation	BMI1, E2F3a, EGFR	[92–94]
miR-137	Decreased	Inhibitor of proliferation, cell differentiation	CDK6	[97]
miR-155	Increased	Regulator of immune response in cells	SMAD2	[97]
miR-181	Decreased	Reduced colony formation and migration	TCL1	[92, 100]
miR-196	Increased	Inductor of proliferation, cell differentiation	HOXB8, HMGA2, ANXA1	[93]
miR-210	Increased	Regulator of proliferation	FGFRL-1	[97]
miR-221	Increased	Cell proliferation	p27Kip1, p57Kip2	[90, 92]
miR-222	Increased	Cell proliferation	p27Kip1, p57Kip2	[90]
miR-296	Increased	Inductor of neovascularization	HGS	[91]
miR-326	Decreased	Reduces cell viability and invasion	NOTCH1/2	[101]
miR-451	Increased	Inhibitor of migration, inductor of proliferation	CAB39	[96]

Potential therapeutic targets and future perspectives

Taking into consideration all the molecular alterations found in glioblastomas, it is clear that the picture of the changes in gliomablastoma becomes more complex as the techniques that enable us to investigate molecular mechanisms develop. The good news is, however, that many of the alterations identified in glioblastoma cluster in three pathways, the P53 (64–87%), RB (68–78%), and the PI3K/AKT (50%), downstream of the receptor tyrosine kinases (altered 88% in total; Fig. 1). Most alterations occur in a mutually exclusive fashion: alterations within one tumor affect only a single gene in a pathway, suggesting that different genes in a pathway are functionally equivalent [7–9, 71].

Quality of models

Functional validation of the identified molecular changes is essential before they can be assessed as targets for therapy. Taking this into account, it becomes clear that good models are needed for high-throughput testing of rationally designed combinations of drugs with specific targets. Several experiments have shown that established glioblastoma cell lines resemble those of the original glioblastomas very poorly when compared at the level of DNA alterations or gene expression profiles [71]. Tumor neurospheres cultured in stem cell medium, organotypic spheroid cultures, or low-passage monolayer cultures, resemble the original tumors better and may be better models for study of glioblastomas in vitro [112, 113].

Therapeutic options, multimodal therapy, and delivery options

For optimum application of the insights presented in this paper, stratified clinical trials are necessary to investigate the best treatment options for each common (group of) genetic alteration(s) in glioblastomas. Ultimately, this could lead to more individualized therapies. Rational drug design and rationally designed clinical trials to test these drugs are needed, because an almost infinite number of compounds is currently available, and these can be tested in limitless numbers of combinations. With genomics approaches, discoveries of common features of different types of tumor may lead to new therapeutic targets and drugs for other tumor types also. The discovery of overexpression of VEGFA and its correlation with poor prognosis in glioblastomas [59] led to trials with the angiogenesis inhibitor bevacizumab. It is currently being used to treat recurrent glioblastoma and phase III trials are being conducted [114, 115].

Rather than single-agent therapy, with which good responses have been obtained in the treatment of other types of cancer but which probably will not suffice in the treatment of glioblastoma, combination treatment is necessary. The clinical response of recurrent glioblastomas to EGFR

inhibitors was found, in one study, to be associated with co-expression of EGFRvIII and PTEN [19] or pAkt [116], but not in combination with TMZ and RT for newly diagnosed glioblastomas [117] or in glioblastomas treated with erlotinib and TMZ [118]. PTEN-deficient glioblastoma patients could, for example, be treated with a cocktail of drugs consisting of an EGFR inhibitor and rapamycin [19], however the results are not yet impressive [119]. The response to TMZ and RT of patients for whom *MGMT* methylation is not observed may be improved by addition of MGMT-depleting agents, which are currently under investigation [120]. In this respect, the choice of anti-epileptic drug may become important as levetiracetam has been shown to inhibit MGMT expression in a preliminary study [121]. In addition, MGMT-mediated TMZ resistance may be overcome by more frequent temozolomide doses in dose-dense schedules [122]. Thus far, the results are disappointing, and a putative disadvantage of combination treatment is the potential increase in side effects [123]. This may, in part, be solved by application of new drug-delivery techniques. In this field, advances have been made with the application of biodegradable wafers, convection-enhanced delivery, and strategically-designed liposomes which circumvent the blood–brain barrier [124, 125]. Recent reviews have provided up-to date overviews on therapy, and we refer the reader to those for more details on ongoing and future therapeutic trials [126].

Synopsis

To summarize, our understanding of the molecular mechanisms underlying subgroups of glioblastoma patients has increased. Moreover, many of the alterations in the aforementioned pathways have been elucidated, and molecular typing of glioblastomas on the basis of gene expression has been used to predict prognosis. Furthermore, for the first time it has been shown that the effects of treatment are distinctly different for different molecular types of glioblastoma classified on this basis [71]. In contrast with many other forms of cancer, however, subsequent application of these results to treatment is lagging behind. Nevertheless, assessment of the molecular profiles of responding versus non-responding patients can be used to determine predictive factors and biomarkers, and may lead to identification of new therapeutic targets. Validation of such new therapeutic approaches will be followed by stratified clinical trials based on such molecular subgroups. Finally, current insights will ultimately lead to more individualized therapy for glioblastoma patients. Combination of current knowledge of molecular alterations in glioblastoma with the availability of many drugs with specific targets makes investigation of new treatments more promising than ever before.

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References

1. Ohgaki H, Kleihues P (2007) Genetic pathways to primary and secondary glioblastoma. Am J Pathol 170:1445–1453
2. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO (2009) Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 10:459–466. doi:10.1016/S1470-2045(09)70025-7
3. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987–996
4. Krex D, Klink B, Hartmann C, von Deimling A, Pietsch T, Simon M, Sabel M, Steinbach JP, Heese O, Reifenberger G, Weller M, Schackert G (2007) Long-term survival with glioblastoma multiforme. Brain 130:2596–2606
5. Burton EC, Lamborn KR, Feuerstein BG, Prados M, Scott J, Forsyth P, Passe S, Jenkins RB, Aldape KD (2002) Genetic aberrations defined by comparative genomic hybridization distinguish long-term from typical survivors of glioblastoma. Cancer Res 62:6205–6210
6. Gorlia T, van den Bent MJ, Hegi ME, Mirimanoff RO, Weller M, Cairncross JG, Eisenhauer E, Belanger K, Brandes AA, Allgeier A, Lacombe D, Stupp R (2008) Nomograms for predicting survival of patients with newly diagnosed glioblastoma: prognostic factor analysis of EORTC and NCIC trial 26981–22981/CE.3. Lancet Oncol 9:29–38
7. TCGAN (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455:1061–1068
8. Yin D, Ogawa S, Kawamata N, Tunici P, Finocchiaro G, Eoli M, Ruckert C, Huynh T, Liu G, Kato M, Sanada M, Jauch A, Dugas M, Black KL, Koeffler HP (2009) High-resolution genomic copy number profiling of glioblastoma multiforme by single nucleotide polymorphism DNA microarray. Mol Cancer Res 7:665–677. doi:10.1158/1541-7786.MCR-08-0270
9. 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 (2008) An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807–1812
10. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674. doi:10.1016/j.cell.2011.02.013
11. Bigner SH, Bjerkvig R, Laerum OD (1985) DNA content and chromosomal composition of malignant human gliomas. Neurol Clin 3:769–784
12. Maxwell JA, Johnson SP, McLendon RE, Lister DW, Horne KS, Rasheed A, Quinn JA, Ali-Osman F, Friedman AH, Modrich PL, Bigner DD, Friedman HS (2008) Mismatch repair deficiency does not mediate clinical resistance to temozolomide in malignant glioma. Clin Cancer Res 14:4859–4868
13. Hunter C, Smith R, Cahill DP, Stephens P, Stevens C, Teague J, Greenman C, Edkins S, Bignell G, Davies H, O'Meara S, Parker A, Avis T, Barthorpe S, Brackenbury L, Buck G, Butler A, Clements J, Cole J, Dicks E, Forbes S, Gorton M, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jenkinson A, Jones D, Kosmidou V, Laman R, Lugg R, Menzies A, Perry J, Petty R, Raine K, Richardson D, Shepherd R, Small A, Solomon H, Tofts C, Varian J, West S, Widaa S, Yates A, Easton DF, Riggins G, Roy JE, Levine KK, Mueller W, Batchelor TT, Louis DN, Stratton MR, Futreal PA, Wooster R (2006) A hypermutation phenotype and somatic MSH6 mutations in recurrent human malignant gliomas after alkylator chemotherapy. Cancer Res 66:3987–3991
14. Dahlback HS, Brandal P, Meling TR, Gorunova L, Scheie D, Heim S (2009) Genomic aberrations in 80 cases of primary glioblastoma multiforme: pathogenetic heterogeneity and putative cytogenetic pathways. Genes Chromosomes Cancer 48:908–924. doi:10.1002/gcc.20690
15. Wiedemeyer R, Brennan C, Heffernan TP, Xiao Y, Mahoney J, Protopopov A, Zheng H, Bignell G, Furnari F, Cavenee WK, Hahn WC, Ichimura K, Collins VP, Chu GC, Stratton MR, Ligon KL, Futreal PA, Chin L (2008) Feedback circuit among INK4 tumor suppressors constrains human glioblastoma development. Cancer Cell 13:355–364
16. Beroukhim R, Getz G, Nghiemphu L, Barretina J, Hsueh T, Linhart D, Vivanco I, Lee JC, Huang JH, Alexander S, Du J, Kau T, Thomas RK, Shah K, Soto H, Perner S, Prensner J, Debiasi RM, Demichelis F, Hatton C, Rubin MA, Garraway LA, Nelson SF, Liau L, Mischel PS, Cloughesy TF, Meyerson M, Golub TA, Lander ES, Mellinghoff IK, Sellers WR (2007) Assessing the significance of chromosomal aberrations in cancer: methodology and application to glioma. Proc Natl Acad Sci USA 104:20007–20012
17. Mulholland PJ, Fiegler H, Mazzanti C, Gorman P, Sasieni P, Adams J, Jones TA, Babbage JW, Vatcheva R, Ichimura K, East P, Poullikas C, Collins VP, Carter NP, Tomlinson IP, Sheer D (2006) Genomic profiling identifies discrete deletions associated with translocations in glioblastoma multiforme. Cell Cycle 5:783–791
18. Ohgaki H, Dessen P, Jourde B, Horstmann S, Nishikawa T, Di Patre PL, Burkhard C, Schuler D, Probst-Hensch NM, Maiorka PC, Baeza N, Pisani P, Yonekawa Y, Yasargil MG, Lutolf UM, Kleihues P (2004) Genetic pathways to glioblastoma: a population-based study. Cancer Res 64:6892–6899
19. Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, Lu KV, Yoshimoto K, Huang JH, Chute DJ, Riggs BL, Horvath S, Liau LM, Cavenee WK, Rao PN, Beroukhim R, Peck TC, Lee JC, Sellers WR, Stokoe D, Prados M, Cloughesy TF, Sawyers CL, Mischel PS (2005) Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 353:2012–2024
20. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Soroceanu L, Williams PM, Modrusan Z, Feuerstein BG, Aldape K (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 9:157–173
21. Pelloski CE, Lin E, Zhang L, Yung WK, Colman H, Liu JL, Woo SY, Heimberger AB, Suki D, Prados M, Chang S, Barker FG III, Fuller GN, Aldape KD (2006) Prognostic associations of activated mitogen-activated protein kinase and Akt pathways in glioblastoma. Clin Cancer Res 12:3935–3941
22. Lopez-Gines C, Gil-Benso R, Ferrer-Luna R, Benito R, Serna E, Gonzalez-Darder J, Quilis V, Monleon D, Celda B, Cerda-Nicolas M (2010) New pattern of EGFR amplification in glioblastoma and the relationship of gene copy number with gene expression profile. Mod Pathol 23:856–865. doi:10.1038/modpathol.2010.62
23. 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 (2009) Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: a prospective translational study of the German Glioma Network. J Clin Oncol 27:5743–5750. doi:10.1200/JCO.2009.23.0805
24. Maher EA, Brennan C, Wen PY, Durso L, Ligon KL, Richardson A, Khatry D, Feng B, Sinha R, Louis DN, Quackenbush J, Black PM, Chin L, DePinho RA (2006) Marked genomic differences characterize primary and secondary glioblastoma subtypes and identify two distinct molecular and clinical secondary glioblastoma entities. Cancer Res 66:11502–11513
25. Holtkamp N, Ziegenhagen N, Malzer E, Hartmann C, Giese A, von Deimling A (2007) Characterization of the amplicon on chromosomal segment 4q12 in glioblastoma multiforme. Neuro Oncol 9:291–297
26. Ichimura K, Vogazianou AP, Liu L, Pearson DM, Backlund LM, Plant K, Baird K, Langford CF, Gregory SG, Collins VP (2008) 1p36 is a preferential target of chromosome 1 deletions in astrocytic tumours and homozygously deleted in a subset of glioblastomas. Oncogene 27:2097–2108
27. Yadav AK, Renfrow JJ, Scholtens DM, Xie H, Duran GE, Bredel C, Vogel H, Chandler JP, Chakravarti A, Robe PA, Das S, Scheck AC, Kessler JA, Soares MB, Sikic BI, Harsh GR, Bredel M (2009) Monosomy of chromosome 10 associated with dysregulation of epidermal growth factor signaling in glioblastomas. JAMA 302:276–289. doi:10.1001/jama.2009.1022
28. Bredel M, Scholtens DM, Yadav AK, Alvarez AA, Renfrow JJ, Chandler JP, Yu IL, Carro MS, Dai F, Tagge MJ, Ferrarese R, Bredel C, Phillips HS, Lukac PJ, Robe PA, Weyerbrock A, Vogel H, Dubner S, Mobley B, He X, Scheck AC, Sikic BI, Aldape KD, Chakravarti A, Harsh GRt (2011) NFKBIA deletion in glioblastomas. N Engl J Med 364:627–637. doi:10.1056/NEJMoa1006312
29. Veeriah S, Brennan C, Meng S, Singh B, Fagin JA, Solit DB, Paty PB, Rohle D, Vivanco I, Chmielecki J, Pao W, Ladanyi M, Gerald WL, Liau L, Cloughesy TC, Mischel PS, Sander C, Taylor B, Schultz N, Major J, Heguy A, Fang F, Mellinghoff IK, Chan TAC (2009) The tyrosine phosphatase PTPRD is a tumor suppressor that is frequently inactivated and mutated in glioblastoma and other human cancers. Proc Natl Acad Sci USA 106:9435–9440. doi:10.1073/pnas.0900571106
30. Nakamura M, Yang F, Fujisawa H, Yonekawa Y, Kleihues P, Ohgaki H (2000) Loss of heterozygosity on chromosome 19 in secondary glioblastomas. J Neuropathol Exp Neurol 59:539–543
31. Bettegowda C, Agrawal N, Jiao Y, Sausen M, Wood LD, Hruban RH, Rodriguez FJ, Cahill DP, McLendon R, Riggins G, Velculescu VE, Oba-Shinjo SM, Marie SK, Vogelstein B, Bigner D, Yan H, Papadopoulos N, Kinzler KW (2011) Mutations in CIC and FUBP1 contribute to human oligodendroglioma. Science 333:1453–1455. doi:10.1126/science.1210557

32. Jenkins RB, Blair H, Ballman KV, Giannini C, Arusell RM, Law M, Flynn H, Passe S, Felten S, Brown PD, Shaw EG, Buckner JC (2006) A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. Cancer Res 66:9852–9861
33. Zheng H, Ying H, Yan H, Kimmelman AC, Hiller DJ, Chen AJ, Perry SR, Tonon G, Chu GC, Ding Z, Stommel JM, Dunn KL, Wiedemeyer R, You MJ, Brennan C, Wang YA, Ligon KL, Wong WH, Chin L, DePinho RA (2008) p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation. Nature 455:1129–1133
34. Pfeifer GP, Hainaut P (2011) Next-generation sequencing: emerging lessons on the origins of human cancer. Curr Opin Oncol 23:62–68. doi:10.1097/CCO.0b013e3283414d00
35. Ekstrand AJ, Sugawa N, James CD, Collins VP (1992) Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. Proc Natl Acad Sci USA 89:4309–4313
36. Lee JC, Vivanco I, Beroukhim R, Huang JH, Feng WL, DeBiasi RM, Yoshimoto K, King JC, Nghiemphu P, Yuza Y, Xu Q, Greulich H, Thomas RK, Paez JG, Peck TC, Linhart DJ, Glatt KA, Getz G, Onofrio R, Ziaugra L, Levine RL, Gabriel S, Kawaguchi T, O'Neill K, Khan H, Liau LM, Nelson SF, Rao PN, Mischel P, Pieper RO, Cloughesy T, Leahy DJ, Sellers WR, Sawyers CL, Meyerson M, Mellinghoff IK (2006) Epidermal growth factor receptor activation in glioblastoma through novel missense mutations in the extracellular domain. PLoS Med 3:e485
37. Knobbe CB, Reifenberger J, Reifenberger G (2004) Mutation analysis of the Ras pathway genes NRAS, HRAS, KRAS and BRAF in glioblastomas. Acta Neuropathol 108:467–470
38. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, Edkins S, O'Meara S, Vastrik I, Schmidt EE, Avis T, Barthorpe S, Bhamra G, Buck G, Choudhury B, Clements J, Cole J, Dicks E, Forbes S, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jenkinson A, Jones D, Menzies A, Mironenko T, Perry J, Raine K, Richardson D, Shepherd R, Small A, Tofts C, Varian J, Webb T, West S, Widaa S, Yates A, Cahill DP, Louis DN, Goldstraw P, Nicholson AG, Brasseur F, Looijenga L, Weber BL, Chiew YE, DeFazio A, Greaves MF, Green AR, Campbell P, Birney E, Easton DF, Chenevix-Trench G, Tan MH, Khoo SK, Teh BT, Yuen ST, Leung SY, Wooster R, Futreal PA, Stratton MR (2007) Patterns of somatic mutation in human cancer genomes. Nature 446:153–158
39. Bleeker FE, Lamba S, Leenstra S, Troost D, Hulsebos T, Vandertop WP, Frattini M, Molinari F, Knowles M, Cerrato A, Rodolfo M, Scarpa A, Felicioni L, Buttitta F, Malatesta S, Marchetti A, Bardelli A (2009) IDH1 mutations at residue p.R132 (IDH1(R132)) occur frequently in high-grade gliomas but not in other solid tumors. Hum Mutat 30:7–11
40. Sanson M, Marie Y, Paris S, Idbaih A, Laffaire J, Ducray F, El Hallani S, Boisselier B, Mokhtari K, Hoang-Xuan K, Delattre JY (2009) Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. J Clin Oncol 27:4150–4154. doi:10.1200/JCO.2009.21.9832
41. Nobusawa S, Watanabe T, Kleihues P, Ohgaki H (2009) IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clin Cancer Res 15:6002–6007. doi:10.1158/1078-0432.CCR-09-0715
42. Ichimura K, Pearson DM, Kocialkowski S, Backlund LM, Chan R, Jones DT, Collins VPC (2009) IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro Oncol 11:341–347. doi:10.1215/15228517-2009-025
43. Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A (2008) Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 116:597–602
44. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, 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 (2009) IDH1 and IDH2 mutations in gliomas. N Engl J Med 360:765–773
45. Bleeker FE, Atai NA, Lamba S, Jonker A, Rijkeboer D, Bosch KS, Tigchelaar W, Troost D, Vandertop WP, Bardelli A, Van Noorden CJ (2010) The prognostic IDH1(R132) mutation is associated with reduced NADP+-dependent IDH activity in glioblastoma. Acta Neuropathol 119:487–494. doi:10.1007/s00401-010-0645-6
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 (2009) Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science 324:261–265. doi:10.1126/science.1170944
47. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM, Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, Su SMC (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 462:739–744. doi:10.1038/nature08617
48. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Xiao MT, Liu LX, Jiang WQ, Liu J, Zhang JY, Wang B, Frye S, Zhang Y, Xu YH, Lei QY, Guan KL, Zhao SM, Xiong Y (2011) Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer Cell 19:17–30. doi:10.1016/j.ccr.2010.12.014
49. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H (2009) IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol 174:1149–1153. doi:10.2353/ajpath.2009.080958
50. Scheurer ME, Etzel CJ, Liu M, El-Zein R, Airewele GE, Malmer B, Aldape KD, Weinberg JS, Yung WK, Bondy ML (2007) Aggregation of cancer in first-degree relatives of patients with glioma. Cancer Epidemiol Biomarkers Prev 16:2491–2495
51. Schwartzbaum JA, Xiao Y, Liu Y, Tsavachidis S, Berger MS, Bondy ML, Chang JS, Chang SM, Decker PA, Ding B, Hepworth SJ, Houlston RS, Hosking FJ, Jenkins RB, Kosel ML, McCoy LS, McKinney PA, Muir K, Patoka JS, Prados M, Rice T, Robertson LB, Schoemaker MJ, Shete S, Swerdlow AJ, Wiemels JL, Wiencke JK, Yang P, Wrensch MRC (2010) Inherited variation in immune genes and pathways and glioblastoma risk. Carcinogenesis 31:1770–1777. doi:10.1093/carcin/bgq152
52. El Hallani S, Marie Y, Idbaih A, Rodero M, Boisselier B, Laigle-Donadey F, Ducray F, Delattre JY, Sanson M (2007) No association of MDM2 SNP309 with risk of glioblastoma and prognosis. J Neurooncol 85:241–244
53. 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, Lonn 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 (2009) Genome-wide association study identifies five susceptibility loci for glioma. Nat Genet 41:899–904. doi:10.1038/ng.407
54. Wrensch M, Jenkins RB, Chang JS, Yeh RF, Xiao Y, Decker PA, Ballman KV, Berger M, Buckner JC, Chang S, Giannini C, Halder C, Kollmeyer TM, Kosel ML, LaChance DH, McCoy L, O'Neill BP, Patoka J, Pico AR, Prados M, Quesenberry C, Rice

T, Rynearson AL, Smirnov I, Tihan T, Wiemels J, Yang P, Wiencke JKC (2009) Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. Nat Genet 41:905–908. doi:10.1038/ng.408
55. Liu Y, Shete S, Etzel CJ, Scheurer M, Alexiou G, Armstrong G, Tsavachidis S, Liang FW, Gilbert M, Aldape K, Armstrong T, Houlston R, Hosking F, Robertson L, Xiao Y, Wiencke J, Wrensch M, Andersson U, Melin BS, Bondy MC (2010) Polymorphisms of LIG4, BTBD2, HMGA2, and RTEL1 genes involved in the double-strand break repair pathway predict glioblastoma survival. J Clin Oncol 28:2467–2474. doi:10.1200/JCO.2009.26.6213
56. IHGSC (2004) Finishing the euchromatic sequence of the human genome. Nature 431:931–945
57. Lal A, Lash AE, Altschul SF, Velculescu V, Zhang L, McLendon RE, Marra MA, Prange C, Morin PJ, Polyak K, Papadopoulos N, Vogelstein B, Kinzler KW, Strausberg RL, Riggins GJ (1999) A public database for gene expression in human cancers. Cancer Res 59:5403–5407
58. Santosh V, Arivazhagan A, Sreekanthreddy P, Srinivasan H, Thota B, Srividya MR, Vrinda M, Sridevi S, Shailaja BC, Samuel C, Prasanna KV, Thennarasu K, Balasubramaniam A, Chandramouli BA, Hegde AS, Somasundaram K, Kondaiah P, Rao MR (2010) Grade-specific expression of insulin-like growth factor-binding proteins-2, -3, and -5 in astrocytomas: IGFBP-3 emerges as a strong predictor of survival in patients with newly diagnosed glioblastoma. Cancer Epidemiol Biomarkers Prev 19:1399–1408. doi:10.1158/1055-9965.EPI-09-1213
59. Godard S, Getz G, Delorenzi M, Farmer P, Kobayashi H, Desbaillets I, Nozaki M, Diserens AC, Hamou MF, Dietrich PY, Regli L, Janzer RC, Bucher P, Stupp R, de Tribolet N, Domany E, Hegi ME (2003) Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. Cancer Res 63:6613–6625
60. Nigro JM, Misra A, Zhang L, Smirnov I, Colman H, Griffin C, Ozburn N, Chen M, Pan E, Koul D, Yung WK, Feuerstein BG, Aldape KD (2005) Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. Cancer Res 65:1678–1686
61. Hormigo A, Gu B, Karimi S, Riedel E, Panageas KS, Edgar MA, Tanwar MK, Rao JS, Fleisher M, DeAngelis LM, Holland EC (2006) YKL-40 and matrix metalloproteinase-9 as potential serum biomarkers for patients with high-grade gliomas. Clin Cancer Res 12:5698–5704
62. Tso CL, Shintaku P, Chen J, Liu Q, Liu J, Chen Z, Yoshimoto K, Mischel PS, Cloughesy TF, Liau LM, Nelson SF (2006) Primary glioblastomas express mesenchymal stem-like properties. Mol Cancer Res 4:607–619
63. Lee Y, Scheck AC, Cloughesy TF, Lai A, Dong J, Farooqi HK, Liau LM, Horvath S, Mischel PS, Nelson SF (2008) Gene expression analysis of glioblastomas identifies the major molecular basis for the prognostic benefit of younger age. BMC Med Genomics 1:52
64. Mir SE, De Witt Hamer PC, Krawczyk PM, Balaj L, Claes A, Niers JM, Van Tilborg AA, Zwinderman AH, Geerts D, Kaspers GJ, Peter Vandertop W, Cloos J, Tannous BA, Wesseling P, Aten JA, Noske DP, Van Noorden CJ, Wurdinger T (2010) In silico analysis of kinase expression identifies WEE1 as a gatekeeper against mitotic catastrophe in glioblastoma. Cancer Cell 18:244–257. doi:10.1016/j.ccr.2010.08.011
65. Tso CL, Freije WA, Day A, Chen Z, Merriman B, Perlina A, Lee Y, Dia EQ, Yoshimoto K, Mischel PS, Liau LM, Cloughesy TF, Nelson SF (2006) Distinct transcription profiles of primary and secondary glioblastoma subgroups. Cancer Res 66:159–167
66. Vitucci M, Hayes DN, Miller CRC (2011) Gene expression profiling of gliomas: merging genomic and histopathological classification for personalised therapy. Br J Cancer 104:545–553. doi:10.1038/sj.bjc.6606031
67. Gravendeel LA, Kouwenhoven MC, Gevaert O, de Rooi JJ, Stubbs AP, Duijm JE, Daemen A, Bleeker FE, Bralten LB, Kloosterhof NK, De Moor B, Eilers PH, van der Spek PJ, Kros JM, Sillevis Smitt PA, van den Bent MJ, French PJ (2009) Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology. Cancer Res 69:9065–9072. doi:10.1158/0008-5472.CAN-09-2307
68. Li A, Walling J, Ahn S, Kotliarov Y, Su Q, Quezado M, Oberholtzer JC, Park J, Zenklusen JC, Fine HAC (2009) Unsupervised analysis of transcriptomic profiles reveals six glioma subtypes. Cancer Res 69:2091–2099. doi:10.1158/0008-5472.CAN-08-2100
69. Bredel M, Scholtens DM, Harsh GR, Bredel C, Chandler JP, Renfrow JJ, Yadav AK, Vogel H, Scheck AC, Tibshirani R, Sikic BI (2009) A network model of a cooperative genetic landscape in brain tumors. JAMA 302:261–275. doi:10.1001/jama.2009.997
70. de Tayrac M, Aubry M, Saikali S, Etcheverry A, Surbled C, Guenot F, Galibert MD, Hamlat A, Lesimple T, Quillien V, Menei P, Mosser J (2011) A 4-gene signature associated with clinical outcome in high-grade gliomas. Clin Cancer Res 17:317–327. doi:10.1158/1078-0432.CCR-10-1126
71. 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 DNC (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17:98–110. doi:10.1016/j.ccr.2009.12.020
72. Esteller M (2008) Epigenetics in cancer. N Engl J Med 358:1148–1159
73. Cadieux B, Ching TT, VandenBerg SR, Costello JF (2006) Genome-wide hypomethylation in human glioblastomas associated with specific copy number alteration, methylenetetrahydrofolate reductase allele status, and increased proliferation. Cancer Res 66:8469–8476
74. Nagarajan RP, Costello JF (2009) Epigenetic mechanisms in glioblastoma multiforme. Semin Cancer Biol 19:188–197. doi:10.1016/j.semcancer.2009.02.005
75. Martinez R, Esteller M (2010) The DNA methylome of glioblastoma multiforme. Neurobiol Dis 39:40–46. doi:10.1016/j.nbd.2009.12.030
76. Nakamura M, Yonekawa Y, Kleihues P, Ohgaki H (2001) Promoter hypermethylation of the RB1 gene in glioblastomas. Lab Invest 81:77–82
77. Nakamura M, Ishida E, Shimada K, Kishi M, Nakase H, Sakaki T, Konishi N (2005) Frequent LOH on 22q12.3 and TIMP-3 inactivation occur in the progression to secondary glioblastomas. Lab Invest 85:165–175
78. Wiencke JK, Zheng S, Jelluma N, Tihan T, Vandenberg S, Tamguney T, Baumber R, Parsons R, Lamborn KR, Berger MS, Wrensch MR, Haas-Kogan DA, Stokoe D (2007) Methylation of the PTEN promoter defines low-grade gliomas and secondary glioblastoma. Neuro Oncol 9:271–279
79. Etcheverry A, Aubry M, de Tayrac M, Vauleon E, Boniface R, Guenot F, Saikali S, Hamlat A, Riffaud L, Menei P, Quillien V, Mosser JC (2010) DNA methylation in glioblastoma: impact on gene expression and clinical outcome. BMC Genomics 11:701. doi:10.1186/1471-2164-11-701

80. Martinez R, Setien F, Voelter C, Casado S, Quesada MP, Schackert G, Esteller M (2007) CpG island promoter hypermethylation of the pro-apoptotic gene caspase-8 is a common hallmark of relapsed glioblastoma multiforme. Carcinogenesis 28:1264–1268
81. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352:997–1003
82. Eoli M, Menghi F, Bruzzone MG, De Simone T, Valletta L, Pollo B, Bissola L, Silvani A, Bianchessi D, D'Incerti L, Filippini G, Broggi G, Boiardi A, Finocchiaro G (2007) Methylation of O^6-methylguanine DNA methyltransferase and loss of heterozygosity on 19q and/or 17p are overlapping features of secondary glioblastomas with prolonged survival. Clin Cancer Res 13:2606–2613
83. Zawlik I, Vaccarella S, Kita D, Mittelbronn M, Franceschi S, Ohgaki H (2009) Promoter methylation and polymorphisms of the MGMT gene in glioblastomas: a population-based study. Neuroepidemiology 32:21–29
84. Christmann M, Nagel G, Horn S, Krahn U, Wiewrodt D, Sommer C, Kaina B (2010) MGMT activity, promoter methylation and immunohistochemistry of pretreatment and recurrent malignant gliomas: a comparative study on astrocytoma and glioblastoma. Int J Cancer 127:2106–2118. doi:10.1002/ijc.25229
85. Brell M, Ibanez J, Tortosa AC (2011) O^6-methylguanine-DNA methyltransferase protein expression by immunohistochemistry in brain and non-brain systemic tumours: systematic review and meta-analysis of correlation with methylation-specific polymerase chain reaction. BMC Cancer 11:35. doi:10.1186/1471-2407-11-35
86. Brandes AA, Tosoni A, Franceschi E, Sotti G, Frezza G, Amista P, Morandi L, Spagnolli F, Ermani M (2009) Recurrence pattern after temozolomide concomitant with and adjuvant to radiotherapy in newly diagnosed patients with glioblastoma: correlation with MGMT promoter methylation status. J Clin Oncol 8:1275–1279
87. Brandes AA, Franceschi E, Tosoni A, Blatt V, Pession A, Tallini G, Bertorelle R, Bartolini S, Calbucci F, Andreoli A, Frezza G, Leonardi M, Spagnolli F, Ermani M (2008) MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. J Clin Oncol 26:2192–2197
88. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW, Aldape KC (2010) Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 17:510–522. doi:10.1016/j.ccr.2010.03.017
89. Filipowicz W, Bhattacharyya SN, Sonenberg N (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 9:102–114. doi:10.1038/nrg2290
90. le Sage C, Nagel R, Egan DA, Schrier M, Mesman E, Mangiola A, Anile C, Maira G, Mercatelli N, Ciafre SA, Farace MG, Agami RC (2007) Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. EMBO J 26:3699–3708. doi:10.1038/sj.emboj.7601790
91. Wurdinger T, Tannous BA, Saydam O, Skog J, Grau S, Soutschek J, Weissleder R, Breakefield XO, Krichevsky AMC (2008) miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. Cancer Cell 14:382–393. doi:10.1016/j.ccr.2008.10.005
92. Ciafre SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G, Negrini M, Maira G, Croce CM, Farace MG (2005) Extensive modulation of a set of microRNAs in primary glioblastoma. Biochem Biophys Res Commun 334:1351–1358
93. Guan Y, Mizoguchi M, Yoshimoto K, Hata N, Shono T, Suzuki SO, Araki Y, Kuga D, Nakamizo A, Amano T, Ma X, Hayashi K, Sasaki T (2010) MiRNA-196 is upregulated in glioblastoma but not in anaplastic astrocytoma and has prognostic significance. Clin Cancer Res 16:4289–4297. doi:10.1158/1078-0432.CCR-10-0207
94. Godlewski J, Nowicki MO, Bronisz A, Williams S, Otsuki A, Nuovo G, Raychaudhury A, Newton HB, Chiocca EA, Lawler S (2008) Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. Cancer Res 68:9125–9130
95. Kim H, Huang W, Jiang X, Pennicooke B, Park PJ, Johnson MDC (2010) Integrative genome analysis reveals an oncomir/oncogene cluster regulating glioblastoma survivorship. Proc Natl Acad Sci USA 107:2183–2188. doi:10.1073/pnas.0909896107
96. Godlewski J, Nowicki MO, Bronisz A, Nuovo G, Palatini J, De Lay M, Van Brocklyn J, Ostrowski MC, Chiocca EA, Lawler SE (2010) MicroRNA-451 regulates LKB1/AMPK signaling and allows adaptation to metabolic stress in glioma cells. Mol Cell 37:620–632. doi:10.1016/j.molcel.2010.02.018
97. Silber J, Lim DA, Petritsch C, Persson AI, Maunakea AK, Yu M, Vandenberg SR, Ginzinger DG, James CD, Costello JF, Bergers G, Weiss WA, Alvarez-Buylla A, Hodgson JGC (2008) miR-124 and miR-137 inhibit proliferation of glioblastoma multiforme cells and induce differentiation of brain tumor stem cells. BMC Med 6:14. doi:10.1186/1741-7015-6-14
98. Kefas B, Godlewski J, Comeau L, Li Y, Abounader R, Hawkinson M, Lee J, Fine H, Chiocca EA, Lawler S, Purow B (2008) microRNA-7 inhibits the epidermal growth factor receptor and the Akt pathway and is down-regulated in glioblastoma. Cancer Res 68:3566–3572
99. Li Y, Guessous F, Zhang Y, Dipierro C, Kefas B, Johnson E, Marcinkiewicz L, Jiang J, Yang Y, Schmittgen TD, Lopes B, Schiff D, Purow B, Abounader RC (2009) MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. Cancer Res 69:7569–7576. doi:10.1158/0008-5472.CAN-09-0529
100. Slaby O, Lakomy R, Fadrus P, Hrstka R, Kren L, Lzicarova E, Smrcka M, Svoboda M, Dolezalova H, Novakova J, Valik D, Vyzula R, Michalek J (2010) MicroRNA-181 family predicts response to concomitant chemoradiotherapy with temozolomide in glioblastoma patients. Neoplasma 57:264–269
101. Kefas B, Comeau L, Floyd DH, Seleverstov O, Godlewski J, Schmittgen T, Jiang J, diPierro CG, Li Y, Chiocca EA, Lee J, Fine H, Abounader R, Lawler S, Purow BC (2009) The neuronal microRNA miR-326 acts in a feedback loop with notch and has therapeutic potential against brain tumors. J Neurosci 29:15161–15168. doi:10.1523/JNEUROSCI.4966-09.2009
102. Huse JT, Brennan C, Hambardzumyan D, Wee B, Pena J, Rouhanifard SH, Sohn-Lee C, le Sage C, Agami R, Tuschl T, Holland ECC (2009) The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. Genes Dev 23:1327–1337. doi:10.1101/gad.1777409
103. Zhang CZ, Zhang JX, Zhang AL, Shi ZD, Han L, Jia ZF, Yang WD, Wang GX, Jiang T, You YP, Pu PY, Cheng JQ, Kang CSC (2010) MiR-221 and miR-222 target PUMA to induce cell survival in glioblastoma. Mol Cancer 9:229. doi:10.1186/1476-4598-9-229

104. Asadi-Moghaddam K, Chiocca EA, Lawler SE (2010) Potential role of miRNAs and their inhibitors in glioma treatment. Expert Rev Anticancer Ther 10:1753–1762. doi:10.1586/era.10.168
105. Deighton RF, McGregor R, Kemp J, McCulloch J, Whittle IR (2010) Glioma pathophysiology: insights emerging from proteomics. Brain Pathol 20:691–703. doi:10.1111/j.1750-3639.2010.00376.x
106. Park CK, Jung JH, Park SH, Jung HW, Cho BK (2009) Multifarious proteomic signatures and regional heterogeneity in glioblastomas. J Neurooncol 94:31–39
107. Iwadate Y, Sakaida T, Hiwasa T, Nagai Y, Ishikura H, Takiguchi M, Yamaura A (2004) Molecular classification and survival prediction in human gliomas based on proteome analysis. Cancer Res 64:2496–2501
108. Furuta M, Weil RJ, Vortmeyer AO, Huang S, Lei J, Huang TN, Lee YS, Bhowmick DA, Lubensky IA, Oldfield EH, Zhuang Z (2004) Protein patterns and proteins that identify subtypes of glioblastoma multiforme. Oncogene 23:6806–6814
109. Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Ponugoti AH, Wiedemeyer R, Stegh AH, Bradner JE, Ligon KL, Brennan C, Chin L, DePinho RA (2007) Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science 318:287–290
110. Du J, Bernasconi P, Clauser KR, Mani DR, Finn SP, Beroukhim R, Burns M, Julian B, Peng XP, Hieronymus H, Maglathlin RL, Lewis TA, Liau LM, Nghiemphu P, Mellinghoff IK, Louis DN, Loda M, Carr SA, Kung AL, Golub TR (2009) Bead-based profiling of tyrosine kinase phosphorylation identifies SRC as a potential target for glioblastoma therapy. Nat Biotechnol 27:77–83
111. Huang PH, Mukasa A, Bonavia R, Flynn RA, Brewer ZE, Cavenee WK, Furnari FB, White FM (2007) Quantitative analysis of EGFRvIII cellular signaling networks reveals a combinatorial therapeutic strategy for glioblastoma. Proc Natl Acad Sci USA 104:12867–12872
112. Lee J, Kotliarova S, Kotliarov Y, Li A, Su Q, Donin NM, Pastorino S, Purow BW, Christopher N, Zhang W, Park JK, Fine HA (2006) Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 9:391–403
113. Li A, Walling J, Kotliarov Y, Center A, Steed ME, Ahn SJ, Rosenblum M, Mikkelsen T, Zenklusen JC, Fine HA (2008) Genomic changes and gene expression profiles reveal that established glioma cell lines are poorly representative of primary human gliomas. Mol Cancer Res 6:21–30
114. ClinicalTrials.gov. A study of Avastin (bevacizumab) in combination with temozolomide and radiotherapy in patients with newly diagnosed glioblastoma. http://clinicaltrials.gov/ct2/show/NCT00943826?term=glioblastoma+bevacizumab&phase=2&rank=2. Accessed 21 October 2011
115. ClinicalTrials.gov. Temozolomide and radiation therapy with or without bevacizumab in treating patients with newly diagnosed glioblastoma. http://clinicaltrials.gov/ct2/show/NCT00884741?term=glioblastoma+bevacizumab&phase=2&rank=1. Accessed 21 October 2011
116. van den Bent MJ, Brandes AA, Rampling R, Kouwenhoven MC, Kros JM, Carpentier AF, Clement PM, Frenay M, Campone M, Baurain JF, Armand JP, Taphoorn MJ, Tosoni A, Kletzl H, Klughammer B, Lacombe D, Gorlia TC (2009) Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. J Clin Oncol 27:1268–1274. doi:10.1200/JCO.2008.17.5984
117. Brown PD, Krishnan S, Sarkaria JN, Wu W, Jaeckle KA, Uhm JH, Geoffroy FJ, Arusell R, Kitange G, Jenkins RB, Kugler JW, Morton RF, Rowland KM Jr, Mischel P, Yong WH, Scheithauer BW, Schiff D, Giannini C, Buckner JC (2008) Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. J Clin Oncol 26:5603–5609
118. Haas-Kogan DA, Prados MD, Tihan T, Eberhard DA, Jelluma N, Arvold ND, Baumber R, Lamborn KR, Kapadia A, Malec M, Berger MS, Stokoe D (2005) Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. J Natl Cancer Inst 97:880–887
119. Reardon DA, Desjardins A, Vredenburgh JJ, Gururangan S, Friedman AH, Herndon JE 2nd, Marcello J, Norfleet JA, McLendon RE, Sampson JH, Friedman HSC (2010) Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma. J Neurooncol 96:219–230. doi:10.1007/s11060-009-9950-0
120. Quinn JA, Jiang SX, Reardon DA, Desjardins A, Vredenburgh JJ, Rich JN, Gururangan S, Friedman AH, Bigner DD, Sampson JH, McLendon RE, Herndon JE 2nd, Walker A, Friedman HSC (2009) Phase II trial of temozolomide plus o6-benzylguanine in adults with recurrent, temozolomide-resistant malignant glioma. J Clin Oncol 27:1262–1267. doi:10.1200/JCO.2008.18.8417
121. Bobustuc GC, Baker CH, Limaye A, Jenkins WD, Pearl G, Avgeropoulos NG, Konduri SDC (2010) Levetiracetam enhances p53-mediated MGMT inhibition and sensitizes glioblastoma cells to temozolomide. Neuro Oncol 12:917–927. doi:10.1093/neuonc/noq044
122. Clarke JL, Iwamoto FM, Sul J, Panageas K, Lassman AB, DeAngelis LM, Hormigo A, Nolan CP, Gavrilovic I, Karimi S, Abrey LEC (2009) Randomized phase II trial of chemoradiotherapy followed by either dose-dense or metronomic temozolomide for newly diagnosed glioblastoma. J Clin Oncol 27:3861–3867. doi:10.1200/JCO.2008.20.7944
123. Kreisl TN, Kim L, Moore K, Duic P, Royce C, Stroud I, Garren N, Mackey M, Butman JA, Camphausen K, Park J, Albert PS, Fine HA (2009) Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. J Clin Oncol 27:740–745
124. Kunwar S, Chang S, Westphal M, Vogelbaum M, Sampson J, Barnett G, Shaffrey M, Ram Z, Piepmeier J, Prados M, Croteau D, Pedain C, Leland P, Husain SR, Joshi BH, Puri RK (2010) Phase III randomized trial of CED of IL13-PE38QQR vs. Gliadel wafers for recurrent glioblastoma. Neuro Oncol 12:871–881. doi:10.1093/neuonc/nop054
125. Madhankumar AB, Slagle-Webb B, Wang X, Yang QX, Antonetti DA, Miller PA, Sheehan JM, Connor JR (2009) Efficacy of interleukin-13 receptor-targeted liposomal doxorubicin in the intracranial brain tumor model. Mol Cancer Ther 8:648–654
126. Clarke J, Butowski N, Chang S (2010) Recent advances in therapy for glioblastoma. Arch Neurol 67:279–283. doi:10.1001/archneurol.2010.5