

Glioblastoma (GB) is highly vascularised tumour, known to exhibit enhanced infiltrative potential. One of the characteristics of glioblastoma is microvascular proliferation surrounding necrotic areas, as a response to a hypoxic environment, which in turn increases the expression of angiogenic factors and their signalling pathways (RAS/RAF/ERK/MAPK pathway, PI3K/Akt signalling pathway and WTN signalling cascade). Currently, a small number of anti-angiogenic drugs, extending glioblastoma patients survival, are available for clinical use. Most medications are ineffective in clinical therapy of glioblastoma due to acquired malignant cells or intrinsic resistance, angiogenic receptors cross-activation and redundant intracellular signalling, or the inability of the drug to cross the blood-brain barrier and to reach its target *in vivo*. Researchers have also observed that GB tumours are different in many aspects, even when they derive from the same tissue, which is the reason for personalised therapy. An understanding of the molecular mechanisms regulating glioblastoma angiogenesis and invasion may be important in the future development of curative therapeutic approaches for the treatment of this devastating disease.

Key words: glioblastoma, signalling pathways, angiogenesis.

Contemp Oncol (Pozn) 2016; 20 (2): 109–118
DOI: 10.5114/wo.2015.56122

New perspectives in glioblastoma antiangiogenic therapy

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Introduction

Glioblastoma (GB), first described in 1926 in the classification of brain tumours by Cushing, is the most common primary brain cancer among adults and the most aggressive malignant brain tumour. GB has an incidence of 1.26 : 1 (men vs. women) and a median survival rate of 13–16 months after standard therapy consisting of maximal resection, radiotherapy, and chemotherapy with temozolomide. Unfortunately, the prognosis is poor with a survival rate of 5% at five years; there is a small increase in the survival rate for patients diagnosed under the age of 20. Only 10% of these types of malignant tumours are secondary neoplasm, evolved from low-grade brain tumours (e.g. anaplastic astrocytoma). The remaining 90% of GB are *de novo* glioblastoma multiforme and have a rapid progression of only three months, compared with the secondary glioblastoma that has a progression time of 4–5 years [1–3]. Glioblastomas are highly vascularised tumours, known to exhibit enhanced infiltrative potential.

Angiogenesis is a central process in cancer progress by forming new capillaries from pre-existing vessels using endothelial cell proliferation, migration, and new lumen organisation, succeeding the signalling of growth factors, proteins, and proteolytic enzymes. A number of studies investigated the different types of angiogenesis implicated in tumour development: sprouting angiogenesis (the first studied form of vasculogenesis), intussusceptive angiogenesis, vasculogenic mimicry, and vessel co-option [4–6].

In recent years, new approaches in chemotherapy have targeted specific receptors such as vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), and epidermal growth factor receptor (EGFR), which are implicated in angiogenesis, tumour cell proliferation, and adhesion. Angiogenic receptors well studied in brain tumour include: EGFR, VEGFR, and PDGFR. VEGFR is involved in glioblastoma progression through several mutations, such as tumour protein p53 (TP53) and protein kinase B/phosphatidylinositol 3-kinase (PIK3R1/PIK3CA). p53 (TP53) and PIK3R1/PIK3CA fail to inhibit VEGF, which in turn stimulate abnormal secretion of VEGF and VEGFR. EGFR overexpression, mainly by EGFRvIII mutation, occurring in 30–70% of primary GB is the most frequent mutation; its overexpression activates EGFR – phosphatidylinositol 3-kinase (PI3K) pathway. Also, new studies offer improved knowledge of these receptors' signalling pathways: mitogen-activated protein kinase signalling (MAPK) pathway and protein kinase B/phosphatidylinositol 3-kinase/mammalian target of rapamycin (AKT/PI3K/mTOR) pathway. AKT/PI3K/mTOR pathway, frequently altered in GB, also with mutation of PTEN gene, makes up for the complexity and heterogeneity of GB, making it one of the most rational targets in GB [7–9]. As a consequence of the last two decades of research in glioblastoma, a better understanding regarding the

tumour's biology, mechanisms to evade apoptosis, and genetic profiling are defined now and are presented as clear targets for GB. Most importantly is the necessity of effective treatment for this aggressive malignancy, molecular therapy being one of the possible solutions.

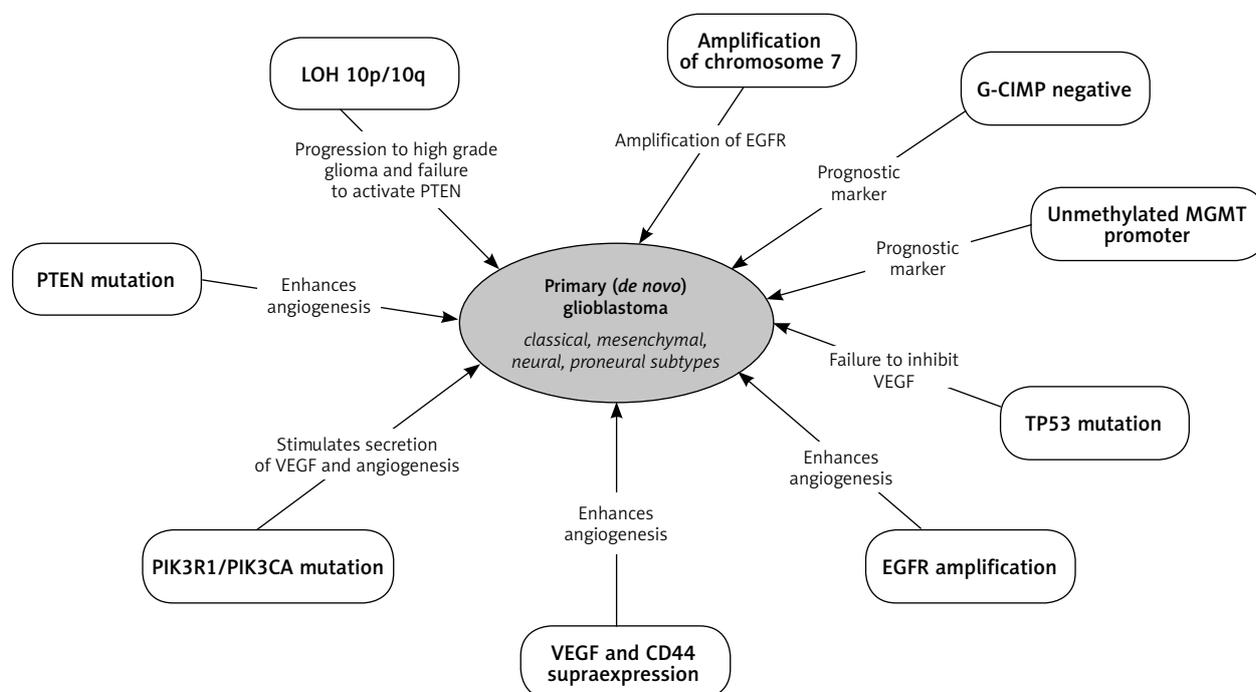
Tumour heterogeneity and angiogenesis in glioblastoma

Angiogenesis is induced early in the stages of development of malignant tumours and is pathologically promoted by a multitude of genetic alterations [10, 11]. Characteristic for angiogenesis in glioblastoma is microvascular proliferation surrounding necrotic areas as a response to a hypoxic environment, which in turn increases the expression of angiogenic factors and their signalling pathways (RAS/RAF/ERK/MAPK pathway, PI3K/Akt signalling pathway and WTN signalling cascade) [12, 13]. Many *in vitro* and *in vivo* studies described and explained the importance of vascular endothelial growth factor (VEGF) system, platelet-derived growth factor (PDGF) system, fibroblast growth factor (FGF) system, insulin-like growth factor-1 (IGF-1) system, angiopoietins, and interleukins as pro-angiogenic factors in GB [14, 15]. All signalling pathways emerging from these molecules (MAPK pathway, PI3K/Akt pathway, and WTN signalling cascade) maintain normal cell proliferation, metabolism, and survival; however, persistent activation of these pathways is correlated with cancer development. Overexpression of angiogenic tyrosine kinase receptors (TKR) is a main factor in the de-

velopment of new vessels, and many modern molecular targeting therapies involve angiogenesis inhibition [16–18]. The various genetic alterations needed for development of primary and secondary glioblastoma and their interaction with tumour angiogenesis are illustrated in Figs. 1 and 2.

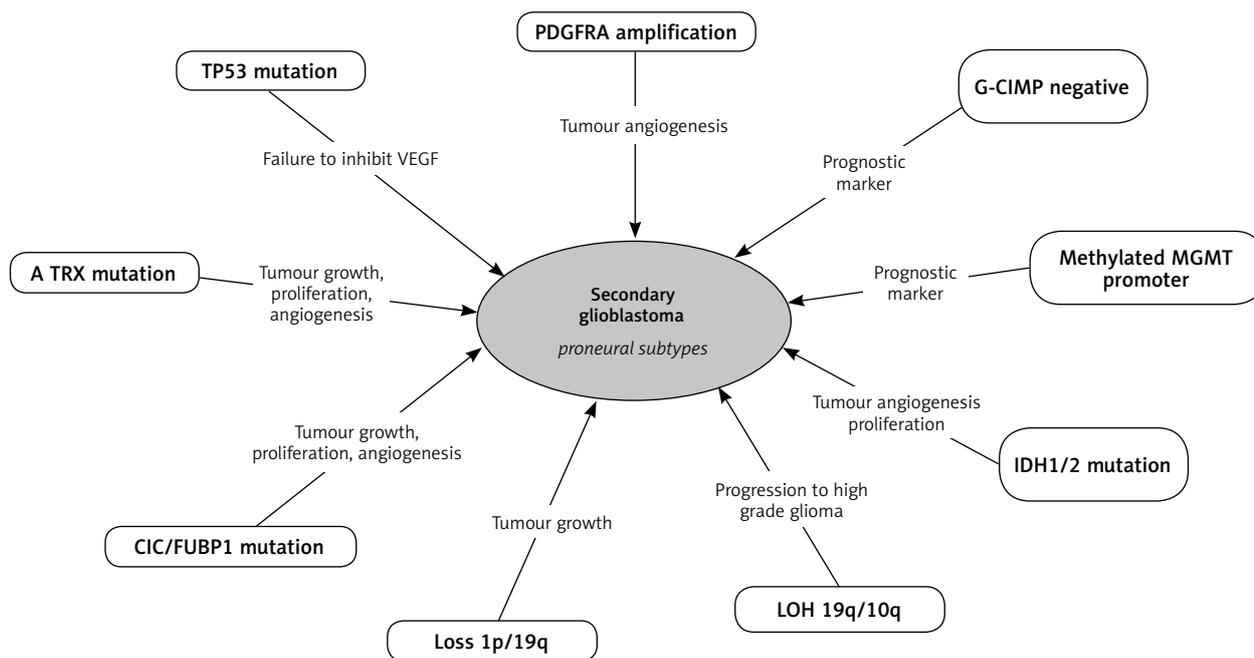
Genetic alterations are critical in developing glioblastoma multiforme, which proved to be a highly heterogeneous tumour. Genomic profiling has been able to differentiate between primary and secondary glioblastoma and identified four subtypes of glioblastoma characterised by different molecular and genetic alterations, all influencing tumour angiogenesis [19, 20].

Genetic expression pattern classifies glioblastoma in classical, mesenchymal, neural and proneural subtypes. Importantly, secondary glioblastoma is predominately formed by the proneural subtype. The first three subtypes described above are known to present IDH-wild type mutation; in contrast, proneural subtype appears to have a high frequency of IDH1/2 (isocitrate dehydrogenase) mutation. Histopathological differentiation was largely indistinguishable until IDH1/2 mutation was proposed for testing, making a definite diagnosis between secondary and primary glioblastoma. IDH1/2 mutation, a biomarker for secondary glioblastoma, is found to be positive in the majority of patients with this type of malignancy. Furthermore, the mutation is present in more than 80% of patients with diffuse and anaplastic astrocytomas, overall being correlated with better prognosis of the disease [21–23]. IDH1/2 mutation along with PTEN mutation (phosphatase and tensin homolog) and EGFR overexpression con-



G-CIMP – CpG island methylation promoter phenotype; LOH 10p/10q – loss of heterozygosity of 10p/10q; PTEN – phosphatase and tensin homolog; VEGF – vascular endothelial growth factor; PIK3R1/PIK3CA – protein kinase B/phosphatidylinositol 3-kinase; TP53 – tumour protein p53; EGFR – epidermal growth factor receptor; MGMT – O6-methylguanine-DNA methyltransferase

Fig. 1. Molecular and genetic alteration in primary glioblastoma development



G-CIMP – CpG island methylation promoter phenotype; *LOH 19q/10q* – loss of heterozygosity of 19q/10q; *TP53* – tumour protein p53; *MGMT* – O6-methylguanine-DNA methyltransferase; *IDH1/2* – isocitrate dehydrogenase 1/2; *CIC/FUBP1* – homolog of *Drosophila capicua/far-upstream binding protein 1*; *ATRX* – α -thalassaemia/mental retardation syndrome X-linked; *PDGFRA* – platelet-derived growth factor receptor A; *VEGF* – vascular endothelial growth factor

Fig. 2. Molecular and genetic alteration in secondary glioblastoma development

tribute to stabilisation of HIF-1 α (hypoxia-inducible factor 1 α) switching to a proangiogenic phase by increasing transcription of VEGF. Non-VEGF pathways that induce angiogenesis in glioblastoma are PDGF, FGF systems, and direct interactions with surface tyrosine kinase receptors. Abnormalities in overexpression of VEGF, CD44, chitinase 3-like 1 (CHI3L1/YKL40), LGALS3 (lectin, galactoside-binding, soluble, 3) genes, TP53 mutations, PDGFRA amplification, PTEN mutation, ATRX mutation (α -thalassaemia/mental retardation syndrome X-linked), loss of heterozygosity of 19q/10q (LOH 19q/10q), loss of chromosome 1p/19q, CIC/FUBP1 (homolog of *Drosophila capicua/far-upstream binding protein 1*) mutation, potent regulators of cell growth, are key factors in the activation of pathologic cellular growth and aberrant angiogenic development [24]. Efforts to map the two different major types of glioblastoma provide not only a good diagnostic tool for prognostic and predictive biomarkers, but also for new and possibly personalised therapeutic targets for patients suffering from this malignancy. IDH1/2 mutation associated with ATRX mutation was significantly correlated with longer survival rates [25]. Moreover, recent observations after genetic profiling of glioblastoma contribute to a better prognosis for patients. One example is the good response to temozolomide and radiotherapy treatment in patients with glioblastoma with MGMT hypermethylation and IDH1/2 mutation [26]. A new study suggests the involvement of YKL40 in progression and drug resistance [27]. Tumour heterogeneity plays a key role in vascular response to treatment by activating secondary pathways for tumour angiogenesis, explaining why targeted therapies such as bevacizumab (a VEGF inhibitor)

or cediranib (a potent VEGFR inhibitor) have such differing results in patients, some having poor response, others presenting with favourable response [28].

Tyrosine kinase receptors and signalling pathways in glioblastoma angiogenesis

In vivo studies reported three VEGFR receptors: VEGFR1 (Flt 1), VEGFR2 (Flk-1/KDR), and VEGFR3 (Flt4), all of them associated with tumour angiogenesis. VEGFR2, the main receptor of the VEGF system, has been reported to be important in GB angiogenesis, Flk1/KDR is present in endothelial cells, and recent research studies have described the secretion of VEGFR2 by GB cells. Flk1 intracellular signalling is mediated through activation of RAS/RAF/ERK/MAPK and PI3K/Akt signalling pathways [29, 30]. The effector proteins are activated after receptor binding to SH2 domain like phospholipase C- δ (PLC δ), usually known to be involved in VEGFR signalling. VEGFR2 activation triggers PI3K and phosphatidylinositol 3,4,5-triphosphate (PIP3), which in turn activates serine/threonine kinase Akt/PKB (protein kinase B). PI3K/Akt signalling pathway is involved in cell proliferation, cell survival, and endothelial cell migration [31–33]. Akt/PKB phosphorylation induces mTOR activation and apoptosis inhibition. mTOR pathway, through p70S6K (p70 ribosomal S6 protein kinase) and 4EBP1(4E-Binding Protein), is known to mediate numerous physiological and pathological processes in angiogenesis, as well as modulating malignant cell proliferation and survival. Akt/PKB also stimulates angiogenesis through endothelial nitric oxide synthesis (eNOS) [34, 35]. One study on GB cells reiterates the importance of PTEN mutation

in PI3K/Akt pathway activation; loss of PTEN triggers the accumulation of PIP3 and Akt activation, which in turn inhibits mTOR pathway. VEGFR activates PLC δ and protein kinase C (PKC), which consecutively activates a series of kinases, including MEK and MAPK. PKC α and PKC β are both involved in tumour progression. Supplementary PKC α , a substrate for PI3K, has properties in modulating cell survival by pro-mitotic and anti-apoptotic actions [7, 15]. In contrast, PKC β is directly involved in angiogenesis, by linking VEGFR2 and evading apoptosis through the interaction with PTEN/Akt. In a randomised clinical trial, one selective inhibitor PKC β showed disappointing results, suggesting the presence of complex interconnections between PI3K/Akt and other signalling pathways, as other compensatory pathways were activated. Tumour proliferation and clonogenicity was shown to be linked to overexpression of VEGFR2, which also seems to act independently of VEGF in GB development [36, 37]. More recently, the expression of VEGFR3 in glioblastomas and haemangioblastomas was also described.

The PDGF family (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, PDGF-DD) have a common growth factor domain named PDGF/VEGF homology domain. Two PDGFRs were reported in the literature: PDGFR α and PDGFR β [14, 38]. PDGFR β uses RAS/RAF/ERK/MAPK signalling pathway for tumour expansion by activating genetic mutations relating to DNA synthesis and mitosis. KRas protein expression occurs frequently in malignancies; however, new research suggests that KRas alone is not sufficient to induce glioblastoma genesis, additional activation of Raf-1, BRAF (serine/threonine-protein kinase B-Raf), ATRX mutation, and TP53 mutation is necessary for glioblastoma oncogenesis [39, 40]. Alone, PDGFR expression acts more as a biomarker for cancer than as a pro-oncogene. PDGFR β is mostly linked to cell motility and proliferation. Autophosphorylation of PDGFR leads to an association between various phosphorylated tyrosine residues, which in turn activate RAS/RAF/ERK/MAPK and PI3K/Akt signalling pathways as well as the signal transduction and transcription activator (STAT) pathway [34, 40, 41]. Some research studies suggest that the involvement of PDGFR in autocrine signalling pathways promotes cancer stem cells in glioblastoma [7, 42, 43].

Another relevant receptor for tumour angiogenesis and expansion is EGFR, which is activated in approximately 50% of primary glioblastomas. This receptor belongs to the ErbB family that consists of four human EGF receptors, Her1-4. The studies for endothelial growth factor receptor denoted a vast manner of pathway signalling: RAS/RAF/ERK/MAPK and PI3K/AKT/mTOR signalling pathway, activation of Wnt/ β -catenin, Notch, and TGF- β (transforming growth factor- β) extensively researched signal pathways [44, 45]. EGFR activation by binding to the ligand activates various molecules like Grb (2 and 7), JAKs (Janus kinases), Src (c-Src tyrosine kinase), PI3K, phospholipase C- δ , SH1, SH2, and STAT; RAS/MAPK signalling pathway, PI3K/AKT/mTOR signalling pathway, after EGFR activation, modulate cell proliferation, differentiation, and survival [41, 46]; JAK/STAT, STAT1, STAT3 or JAK-independent activation trigger on transcription factors such as c-jun, c-fos, jun B and c-Myc.

EGFR also interacts with Wnt/ β -catenin pathway at various points. Wnt/ β -catenin pathway plays a significant role in the survival of several types of tumour cells, including brain tumours, making this pathway, in recent years, an important target for glioblastoma therapy. In cancer, interaction of Wnt and Frizzled/lipoprotein-receptor related protein (Fzd) generates the formation of the Dvl-Fzd complex (Dishevelled-Frizzled complex) that inactivates regulatory mechanisms including leukocyte enhance factor-1 (LEF-1) or T cell factors (TCF). TGF- β is frequently upregulated in glioblastoma, acting through the Smad (Mad-homologues, MADH) transcription factors family and receptor-regulated Smads (R-Smad). TGF- β pathway is implicated in glioma invasiveness and migration. Notch pathway is fundamental to normal development, and its deregulation is involved in tumour angiogenesis, cell proliferation, and apoptosis. EGFR is able to modulate both TGF- β and Notch pathways, the latter being influenced in a smaller proportion by VEGFR [5, 47–49]. Tumour apoptosis is induced by the selective inhibitors of angiogenesis, tumour proliferation, and inhibition of pathways signalling and is facilitated by activation of caspase cascade (cysteine inyl aspartate proteinases) [50].

FGFR, activated by FGF ligand, modulates a series of processes, including cell proliferation and cell migration. Through GAB1 (GRB2-associated binding protein 1), SOS1 (Son of sevenless homolog 1), SHC1 (Src homology 2 domain containing transforming protein 1) and Grb2 domains FGF1-FGFR activates RAS/RAF/ERK/MAPK signalling pathway. New evidence shows that FGFR is involved in a number of pro-oncogenic processes in GB such as tumour invasion and proliferation, being correlated with a poor prognostic in patients with GB [51]. PI3K/AKT/mTOR signalling pathway activation by FGFR is important in cell survival and angiogenesis; a recent study strongly related their ligand, FGF2, as a prognostic biomarker, to the proneural type of glioblastoma [8]. FGF1-FGFR also activates other pathways such as Jnk/p38 Mapk and STAT3/ NF- κ B, with crucial implications in neurogenesis, apoptosis, cell proliferation, and invasion [52]. VEGF and FGF autocrine feedback loop has been shown to increase supra-activation of their cognate receptors and mediate tumour growth. A schematic overview of different signalling pathways involved in tumour cell proliferation, migration, and survival is depicted in Fig. 3.

Drug development in glioblastoma

The new molecular targeted therapies focus on the angiogenic TRKs and their signalling pathways inactivation. In 2009, the Food and Drug Administration (FDA) approved bevacizumab (Avastin), the first drug from the emerging class of new molecular therapy, as a second-line treatment of recurrent glioblastoma. Bevacizumab is also used as a VEGF inhibitor in other types of neoplasm: breast, lung, and colon [53, 54]. Nowadays, more molecular-specific drugs are being evaluated in clinical studies: cediranib, a potent VEGFR inhibitor (REGAL trial), sunitinib (Sutent), a multikinase inhibitor for VEGFR, PDGFR, c-Kit (tyrosine-protein-kinase KIT), sorafenib (Nexaver), and a multikinase inhibitor for VEGFR, PDGR, c-Kit, and for

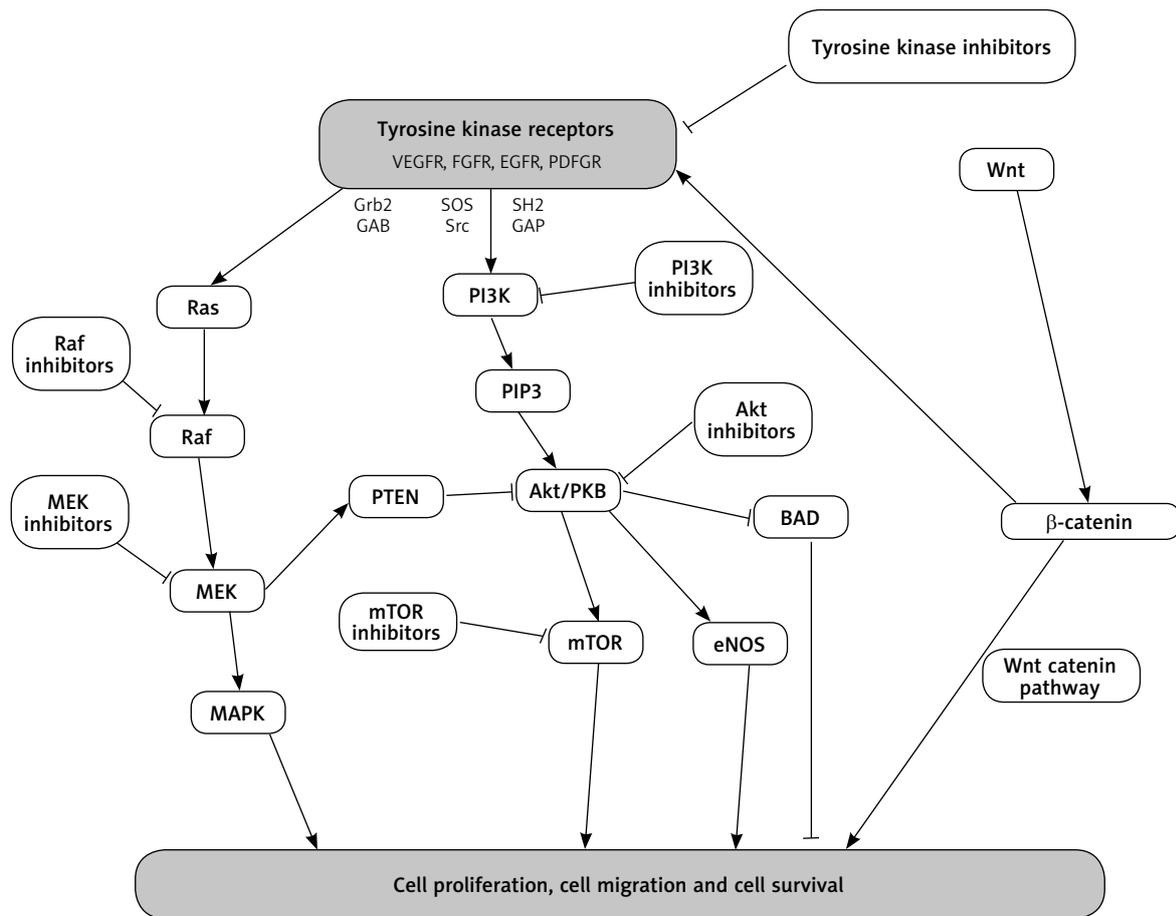


Fig. 3. Tyrosine kinase receptors and their signalling pathways involved in angiogenesis. Wnt catenin pathway stimulates tyrosine kinase receptors (TKRs) activation. RAS/RAF/ERK/MAPK and PI3K/Akt signalling pathways activation can produce a variety of processes involved in angiogenesis like cell proliferation, migration and survival. eNos (endothelial nitric oxide synthase) influenced by AKT/PKB promotes vascular permeability. MEK stimulates PTEN (phosphatase and tensin homolog) inhibiting protein kinase B (PKB). BAD (Bcl-2-associated death promoter); Potential therapeutic targets are specified

RAS/RAF/ERK/MAPK signalling pathway [55–57]. Despite the new advances in drug discovery, the majority of newly targeted therapies are still in clinical trial phase, whether alone or in combination with standard chemotherapeutics (temozolomide, carboplatin, cisplatin, lomustine) or radiotherapy [37, 58]. A novel discovery is the better outcome of patients with hypermethylation of O⁶-methylguanine-DNA methyltransferase (MGMT) gene promoter, presently believed to confer tumour chemosensitivity. MGMT gene promoter has been described in approximately 50% of glioblastoma multiforme.

VEGF system remains an important target for the new molecular therapies. One example is VEGF Trap (aflibercept), acting as competitive VEGF-A isoforms binding to VEGFR1/2, with good effect on tumour cells but high host toxicity [59]; the capacity of VEGFR to act independently in GB has changed the perspective in GB treatment, making the receptors TKR a more rationale target. VEGFR2 inhibitors, such as Avastin (CT-322), showed promising results in a phase I clinical trial [60]. BEZ 235, a dual PI3K/mTOR inhibitor, had favourable outcomes in a phase I and II clinical trial, being well tolerated in patients with solid tumour

and prolonging the patients' survival [61, 62]. Apoptosis in cancer cells treated with BEZ235 is mediated through extrinsic pathway by activation of TNF-R1 (ligand-activated tumour-necrosis factor receptor-1), FAS (Apo-1), DR4/DR5 (TRAIL receptors 1/2) that bind TNF α , CD95, and tumour necrosis factor-related apoptosis inducing factor (TRAIL) and activates caspase cascade through caspase 2. One *in vitro* study showed that responders to dual PI3K/mTOR inhibitor contained EGFR amplification or PI3K mutation [63]. Conversely, drug resistance is one of the major causes for failure of response to treatment, and it seems that ERK pathway activation is one of the main factors of drug resistance [64, 65]. Another dual PI3K/mTOR inhibitor, XL 765, acts by inhibition of phosphorylation of multiple PI3K/phosphatase pathways proteins [66]. Congruent results were found in one study involving a PI3K/AKT inhibitor, BKM120 [67]. Despite unsure results in a phase I clinical trial for XL 765 due to adverse effects on liver function, an interesting phenomenon was the lack of apoptosis induction through caspase cascade; the antiproliferative property was associated with G1 phase specific block [68]. An important problem in therapy failure is the continuous ability

Table 1. Molecular therapy currently in preclinical and clinical development

Agent	Mechanism of action	Phase development in glioblastoma	Indication for other cancers
Cetuximab [70]	EGFR	phase II trial in patients with recurrent GB	colon cancer
Erlotinib [71, 72]	EGFR	phase II trial in patients with newly diagnosed GB; phase I/II trial in patients with recurrent diagnosed GB in combination with temsirolimus	NSCLC, pancreatic cancer
Nimotuzumab [73]	EGFR	phase III trial in patients with newly diagnosed GB	pancreatic cancer
Angiocept (CT-322) [74]	VEGFR-2	phase II trial in patients with recurrent diagnosed GB (insufficient efficacy)	solid tumours
ABT-414 [75]	active EGFR or mutant EGFRvIII	phase I trial in patients with recurrent unresectable GB	solid tumours, NSCLC, prostate cancer, colorectal cancer
Gefitinib [76]	EGFR	phase I/II trial in patients with newly diagnosed GB in combination with radiation therapy	advanced or metastatic NSCLC, breast cancer
Aflibercept [77]	anti-VEGF	phase II trial in patients with recurrent diagnosed GB	metastatic colorectal cancer
Vatalanib [78]	VEGFR, PDGFR, and c-KIT	phase I trial in patients with newly diagnosed GB in combination with standard therapy	metastatic colorectal cancer
Dasatinib [79]	SRC, c-KIT, EPHA2, and PDGFR	phase II trial in patients with recurrent GB	CML, ALL
Sunitinib [80]	VEGFR 2, PDGFR, c-KIT, FLT3	phase II trial in patients with recurrent GB	gastrointestinal tumour, renal cell carcinoma
Sorafenib [81, 82]	VEGFR 1-2, PDGFR α - β , c-KIT, FLT3, and RET	phase I trial in patients with newly diagnosed GB in combination with standard therapy; phase II trial in patients with recurrent GB	renal cell carcinoma, renal tumours, hepatocellular carcinoma
Motesanib [83]	VEGFR, PDGFR, c-KIT	–	NSCLC
Vandetanib [84, 85]	VEGFR2, EGFR, and RET	phase II trial in patients with newly diagnosed GB and phase I/II trial in patients with recurrent GB	thyroid cancer, NSCLC
Pazopanib [86]	VEGFR-1-2 -3, PDGFR α - β , c-KIT	phase II trial in patients with recurrent GB	renal tumour, sarcoma
Bosutinib [87]	Src and ABL	phase II trial in patients with recurrent GB	
Nilotinib [88]	ABL1/BCR-ABL1 and KIT, PDGFR	–	metastatic gastrointestinal stromal tumours
Axitinib [89]	VEGFR-1, -2, -3	proved efficient in preclinical models of glioblastoma	melanoma, NSCLC
Bez 235 [90]	PI3K/mTOR	proved efficient in preclinical models of glioblastoma	solid tumours, metastatic breast cancer
Everolimus [91]	mTOR	phase II trial in patients with newly diagnosed GB in combination with standard therapy	renal cell carcinoma, lymphoma, hepatocellular carcinoma
Temsirolimus [72, 92]	mTOR	phase II trial in patients with recurrent GB in combination with bevacizumab; phase I/II trial in patients with recurrent diagnosed GB in combination with erlotinib	solid tumours, haematological malignancies
BKM120 [93]	PI3K/Akt	proved efficient in <i>in vitro</i> models of glioblastoma	solid tumours, NSCLC, prostate cancer, colorectal cancer, haematological malignancies
XL 184 [94]	MET, VEGFR-2 and RET, KIT	phase II trial in patients with progressive/recurrent GB	medullary thyroid cancer, NSCLC
XL 765 [68]	PI3K, mTOR	phase I trial in patients with recurrent/GB	solid tumours, NSCLC
SF 1126 [95]	PI3K, mTOR	proved efficient in preclinical models of glioblastoma	solid tumours

Table 1. Cont.

PF 4691502 [96]	PI3K, mTOR	proved efficient in <i>in vitro</i> models of glioblastoma	colorectal cancer, breast and gastric cancer
Perifosine (KRX-0401) [97]	AKT	phase I/II trial in patients with recurrent GB; phase II trial in patients with recurrent GB in combination with temsirolimus (ongoing)	colorectal cancer, MM, NSCLC, renal cell carcinoma, ovarian cancer and haematological malignancies
AZD 2014 [98]	mTOR	proved efficient in <i>in vitro</i> models of glioblastoma stem like cells enhances radiosensitivity	solid tumours
Celgene (CC-223) [99]	mTOR	proved efficient in preclinical models of glioblastoma	NHL and MM
INK 128 [100]	mTOR	recruiting phase in patients with recurrent GB	NHL, MM, solid tumours

of the tumour to develop drug resistance mechanisms, observed after new molecular targeted therapy in many phase II/III clinical trials. Evolving theories are stating the importance of tumour heterogeneity and its correlation with angiogenesis, which are important factors for continuum disease progression and evasion from new targeted therapies in glioblastoma. Table 1 highlights the different development phases for small molecular targeted drugs in glioblastoma [16, 29, 35, 69–100].

Several signalling pathway inhibitors, such as mTOR inhibitors everolimus and temsirolimus, showed dissatisfactory results in clinical trials, in part due to rapid progression of drug resistance [101]. Everolimus, a specific inhibitor of mTOR, is known to activate compensatory pathways, although favourable results were depicted in a few preclinical and clinical studies when used in combination with bendamustine or radiotherapy. Apoptosis pathways are activated through PI3K/AKT/PKB system downstream inducing phosphorylation of mTOR, not blocked by specific mTOR inhibitors [102, 103]. Perifosine (KRX-0401), an AKT pathway inhibitor, was found to induce cytotoxicity and evade drug resistance in a study regarding myeloma. Inhibition of AKT through activation of anti-apoptotic signals is one response for obtaining drug efficacy. Another response is activation of JNK (c-Jun N-terminal kinases) pathway, which is a known byway implicated in cellular apoptosis [104, 105]. Increasing the perifosine concentration was demonstrated to have an amplified effect on cellular apoptosis. Apoptosis in correlation with treatment occurs through caspase cascade, particularly through caspase-3 and -9 [106].

Limited clinical benefit was reported after forerlotinib, gefitinib, or cetuximab in recurrent or primary glioblastoma, either as monotherapy or in combination with other treatment approaches for glioblastoma. Multi-targeted inhibitor drugs are expected to be more effective compared to those that inactivate a single receptor. One example is the case of sorafenib, a multikinase inhibitor, with consistently good outcomes in clinical trials [107, 108]. Sorafenib induces apoptosis by targeting Mcl-1 protein, an antiapoptotic member of BCL2 family, in turn reducing cell viability. [109]. Similarly dasatinib, a SRC inhibitor (proto-oncogene tyrosine-protein kinase), known as an inhibitor of Bcr-Abl tyrosine kinase), has promising results by inhibiting cell

invasion, sustaining apoptosis and tumour regression in glioblastomas and other malignant diseases. Apoptosis is induced by downregulation of PI3K/AKT and STAT3, which act by compromising PTEN function and Src inhibition. Loss of both PTEN and p53 induces primary glioblastoma, with devastating pathological and clinical effects. In vitro studies with dasatinib showed efficacy of apoptosis on EGFR-mutant cells sensitive to gefitinib, but with minimal effect on WT EGFR cells [110]. Dasatinib has an inhibitory effect also on PDGFR and c-Kit [111]. Disappointing results were also obtained in a clinical trial for glioblastoma patients with the multi-kinase inhibitor imatinib, a PDGFR, KIT, and ABL-kinase inhibitor, despite the success obtained in treating certain types of leukaemia [112]. Significant evidence of drug resistance mechanism is MET (MET proto-oncogene, receptor tyrosine kinase) amplification, detected after treatment with various tyrosine kinase inhibitors such as erlotinib, gefitinib, and imatinib. MET amplification is frequently associated with poor prognosis considering several biologic processes that initiate invasive growth. MET activation is induced by a hypoxic state, producing a high sensitivity to HGF (hepatocyte growth factor), activating RAS/ MAPK, PI3K/AKT and STAT3 pathways that will induce the so-called “invasive switch” [113, 114].

Based on the genetic and molecular pathology of glioblastoma, researchers provided new insight in treating this disease. EGFR amplification and PTEN mutation are known to induce drug resistance after treatment with EGFR inhibitors [115]. The pathological EGFR expression in glioblastoma is proposed to activate all intracellular mitogenic signalling (PI3K/AKT/mTOR, Raf/MEK/ERK, and Src/STAT pathways) by interacting not only through Grb, JAKs, Src, PI3K, phospholipase C- δ , SH1, SH2, and STAT but also through ErbB2 and ErbB3 transactivation [35, 65].

Conclusions and perspectives

Researchers have observed that GB tumours are different in many aspects, even when they derive from the same tissue, providing a reason for personalised therapy. An understanding of the molecular mechanisms regulating GB angiogenesis and invasion may be important in development of curative therapeutic approaches for the treatment of this devastating disease.

The authors declare no conflict of interest.

The research is financed by: "Program of Excellence in multidisciplinary doctoral and postdoctoral research in chronic diseases", Contract No. POSDRU/159/1.5/S/133377, co-financed project from the European Social Fund by Operational Sectoral Programme Human Resources Development 2007–2013 and PN-II-ID-PCE-2011-3-1041 UEFISCDI, Romania.

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Submitted: 26.05.2015

Accepted: 15.10.2015