Primary cilium and glioblastoma

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Abstract: Glioblastoma (GBM) represents the most common, malignant and lethal primary brain tumour in adults. The primary cilium is a highly conserved and dynamic organelle that protrudes from the apical surface of virtually every type of mammalian cell. There is increasing evidence that abnormal cilia are involved in cancer progression, since primary cilia regulate cell cycle and signalling transduction. In this review, we summarize the role of primary cilium specifically with regard to GBM, where there is evidence postulating it as a critical mediator of GBM tumorigenesis and progression. This opens the way to the application of cilia-targeted therapies (‘ciliotherapy’) as a new approach in the fight against this devastating tumour.

Keywords: ciliogenesis, glioblastoma, glioma stem cells, molecular mechanism, primary cilium

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Introduction
Glioblastoma (GBM) is a grade IV diffuse glioma according to the current criteria established by the World Health Organization (WHO)1 and represents the most common, malignant and lethal primary brain tumour in adults. Indeed, GBM accounts for about 50% of all gliomas and 45–50% of malignant primary brain and central nervous system (CNS) tumours, with an age-adjusted incidence ranging from 0.59 to 3.69 cases per 100,000 individuals.2,3 Because of its highly invasive nature, rapidly infiltrating the surrounding brain parenchyma, GBM has poor prognostic and survival rates with a median overall survival of 15 months and a 5-year survival rate of less than 5%,2 despite aggressive treatment combining surgery, radiotherapy and temozolomide (TMZ)-based chemotherapy.4 From a clinical perspective, two major GBM subtypes can be defined: primary or de novo GBMs comprise around 90% of total GBMs and mainly affect patients over 55 years old without any prior finding, whereas secondary GBMs develop in younger individuals from a previous lower-grade glioma and carry better prognosis.5 Nonetheless, the genomic and transcriptomic data obtained by The Cancer Genome Atlas (TCGA)6,7 have led to a molecular stratification of GBM based on genetic abnormalities and signalling pathways affected, establishing four subtypes: classical, mesenchymal, proneural and neural.7,8 Thus, classical GBMs are mainly defined by high-level EGFR amplification and overexpression, PTEN and CDKN2A deletion, absence of TP53 mutations, high expression of the neural/stem cell marker NES and activation of Sonic hedgehog (Shh) and Notch pathways. On its part, the mesenchymal subtype shows loss of NF1 mutations in PTEN and NF1, expression of mesenchymal markers such as CHI3L1 and MET, activation of mitogen-activated protein kinase (MAPK) signalling and high expression of genes involved in tumour necrosis factor (TNF) and nuclear factor kappa B (NF-κB) pathways. Proneural GBMs are characterized by amplification, overexpression and mutation of PDGFRA, mutations in IDH1 and TP53, expression of genes involved in proneural (such as SOX gene family) and oligodendrocytic development, and activation of the phosphatidylinositol-3-kinase (PI3K) pathway. Finally, neural GBM lacks a particular genetic profile but distinctively expresses neuronal markers such as NEFL, GABRA1, SYT1 and SLC12A5. Although clinical and molecular subtypes do not fully overlap, a significant correlation has been established between primary GBMs and the classical subtype, on the one hand, and...
secondary GBMs with the proneural subtype, on the other; however, the presence or absence of IDH1/2 mutations is the only diagnostic molecular marker considered by WHO to sub-classify GBM, so that primary GBMs are IDH-wildtype while IDH-mutated tumours correspond closely to secondary GBM. Furthermore, differences in prognosis and response to treatment have been described for these molecular subtypes, with proneural GBM displaying better prognosis and improved survival that are not related to the first-line multimodal treatment, which correlates with a delayed mortality in both classical and mesenchymal subtypes. The better outcome observed in proneural GBM is probably due to the presence of widespread hypermethylation across the genome, the so-called glioma-CpG island methylator phenotype or G-CIMP, which is tightly associated with the presence of IDH1/2 mutations. In this regard, MGMT promoter methylation has also been established as a good predictive marker for treatment response to alkylating chemotherapeutic agents such as TMZ, but recent work links this finding only to the classical GBM subtype.

Unfortunately, GBM is currently considered incurable due to several intrinsic factors. First, the high morphological, cellular, molecular and genetic heterogeneity reported, mostly at intratumoral level, is believed to be a crucial factor for therapy failure and tumour relapse in GBM. Second, and even more importantly, GBMs harbour a small population of highly tumorigenic, self-renewing glioma stem cells (GSCs) that decisively contribute to tumour initiation, maintenance and recurrence, and also to radio- and chemotherapy resistance. There is therefore an urgent need to develop further research in GBM pathogenesis in order to identify new potential targets for treatment. In this sense, several recent works have addressed the role of primary cilia and ciliogenesis in GBM, which could represent an important niche scarcely explored until now.

The primary cilium, also known as immotile or sensorial cilium, is a highly conserved and dynamic organelle that protrudes from the apical surface of virtually every type of mammalian cell in a single copy. Primary cilia are composed of nine microtubule doublets without a central pair (9+0 structure) that form the ciliary axoneme, which is surrounded by a bilayer lipid membrane that is continuous with the plasma membrane but enriched in specific proteins and lipids required for ciliary activity; this particular composition is maintained through a specialized region at the proximal axoneme named the transition zone, which acts as a ciliary gate (see Figure 1).

The axoneme is nucleated by the centrosomal mother centriole, which migrates to the ciliary assembly site and turns into the basal body of the mature primary cilium. The success of a correct cilia elongation and function relies on a bidirectional transport system called intraflagellar transport (IFT), which carries cilia-targeted cargoes in and out of the cilium by two large protein complexes (IFT particles). Once these particles are assembled, they move onto the microtubules along the axoneme either to the tip (anterograde transport) or to the base (retrograde transport) of the cilium in a process mediated by kinesin-2 and cytoplasmic dynein-2 motors, respectively (Figure 1). The primary cilium is widely considered the sensorial antenna of the cell, acting as a central hub for coordinating most of cellular signalling pathways such as Shh, Wingless/int (Wnt), transforming growth factor beta (TGF-β), Notch and mechanistic target of rapamycin kinase (mTOR), as well as signalling cascades mediated by receptor tyrosine kinases (RTKs), G-protein coupled receptors (GPCRs), purinergic receptors, ion channels or receptors for extracellular matrix proteins. Thus, by means of a receptor-enriched ciliary membrane, primary cilia are able to capture extracellular signals and integrate them to elaborate biological responses that involve cell cycle control, development and differentiation processes, migration and polarity, or proliferation and maintenance of stem cells. Focusing on the brain, the primary cilium activity is pivotal to regulate several stages of neurogenesis including early brain patterning, proliferation and differentiation of neural progenitor/stem cells, and neuronal maturation and maintenance. In fact, neuronal cilia are abundant and have a widespread distribution in the brain, which combined with the neuronal ciliary membrane enrichment in signalling elements such as GPCRs [somatostatin receptor 3 (SSTR3), melanin concentrating hormone receptor 1 (MCHR1), 5-hydroxytryptamine receptor 6 (HTR6), among others] and downstream signalling molecules [adenylate cyclase 3 (ADCY3)], suggests neuronal cilia can mediate neurotransmitter and hormone-mediated signalling to modulate neural activity. However, little is known about the role of primary cilia in glial cells (see below).
Defects in structure and/or function of cilia is the common aetiology of an expanding and heterogeneous group of inherited disorders collectively termed ciliopathies, mainly affecting primary cilium. Remarkably, many ciliopathies display neurological features (e.g. cognitive impairment, developmental delay, ataxia, brain malformations) and obesity, which have been related to cilia loss and mislocalization of ciliary receptors in hypothalamic neurons; these findings highlight that defects in neuronal cilia could lead to disturbed signalling pathways that disrupt brain homeostasis and result in disease phenotypes. Furthermore, there is increasing evidence that abnormal cilia are likely involved in cancer progression, since primary cilia regulate cell cycle and signalling transduction. On the one hand, ciliogenesis is tightly coupled to the cell cycle, so ciliary disassembly is a requisite to liberate the centriole and thereby promote mitotic spindle formation; primary cilium is in fact considered as a structural checkpoint for cell cycle re-entry. In this sense, loss or decrease of cilia is a commonly reported feature in different malignant tumours such as breast cancer, pancreatic adenocarcinoma, melanoma or renal cell carcinoma, so primary cilium has been proposed to act as a tumour suppressor organelle. On the other hand, many cancers display alterations in different signalling cascades, especially involving the upregulation of Shh signalling, which in turn constitutes the best-characterized cilia-regulated pathway. Finally, the emerging interplay between cilia and autophagy, a proteolytic mechanism essential to maintain homeostasis that is deregulated in cancer, represents additional evidence that strongly links cilia and cancer.

**Linking primary cilium and glioblastoma: current evidence**

The role of primary cilium in glial cells, and more specifically with regard to astrocytes as GBM is a high-grade astrocytic malignancy, has been...
addressed in several works over the last years. Although much less is known compared with neuronal primary cilia, there is widespread evidence that glial cells possess a functional primary cilium, both in vivo and in cultured glial cells, which has been recently characterized. 

Structurally, only small populations of astrocytic cilia are ADCY3-positive, unlike neuronal primary cilia, which strongly associate with ADCY3 expression; conversely, the primary cilium assembled by astrocytes is better marked by ADP ribosylation factor-like GTPase 13B (ARL13B), a well-known ciliary protein of the Arf-like small GTPase family involved in ciliogenesis and ciliary trafficking. Concerning the functional implications of astrocytic primary cilia, these organelles have been mainly involved in controlling the expansion of radial astrocytes, considered the adult neural stem cells. Thus, radial astrocytes develop a primary cilium that is required to mediate the Shh-dependent proliferation and/or maintenance of these progenitors in adult hippocampus, as well as in the ventricular–subventricular zone, the major CNS postnatal germinal niche. Furthermore, primary cilia was described to act as the central hub that integrates and transduces the Shh signalling in astrocytes in order to regulate cell survival under stress conditions.

Remarkably, key components of the Shh machinery such as the transducer Smo, frizzled class receptor (SMO) or the membrane receptor Patched 1 (PTCH1) localize to astrocytic primary cilia. Early work describing ciliated cells in human astrocytoma biopsies dates from the early 1970s, though it was not until 2009 that Moser and colleagues reported the first comprehensive comparison of ciliary expression profiles between normal human astrocytes and five human astrocytoma/GBM cell lines (U-87 MG, T98G, U-251 MG, U-373 MG and U-138 MG). This pioneering study proposed for the first time that aberrant ciliogenesis is a common feature in GBM-derived cells and it may contribute to developing malignant phenotypes. In detail, primary cilium was disrupted at early stages so that fully formed cilia were either completely absent, extremely rare or abnormal after incomplete ciliogenesis. In this sense, significant differences depending on the specific cell line were reported – for example, only U-87 MG cells were able to assemble an immature axoneme (with a frequency less than 1%) while the T98G cell line was never documented to initiate ciliogenesis, with the remaining lines displaying cilia in intermediate stages. In addition, centriolar findings such as abnormally shorter or longer centrioles as well as structural anomalies in distal/sub-distal appendages were also reported. Further work by the same authors corroborated these results in human GBM biopsies, with six of the seven patients analysed showing the previously reported defects in ciliogenesis, although extensive patient heterogeneity was noted. Based on these two descriptive studies, Moser and colleagues pointed out that abnormal primary cilia constitute a hallmark of GBM pathology. In contrast to the latter studies, a larger analysis of 23 GBM biopsies and five primary human GBM cell lines showed that small subpopulations in all cells/biopsies have mature primary cilia (8–25% of the total in each cell line/biopsy), but non-ciliated cells with abnormal centrioles were also identified. Moreover, cilia were observed all across the tumour microenvironment in the case of GBM tumours, including necrotic areas and the vasculature vicinity. Remarkably, these primary cilia are likely functional since well-known ciliary trafficking proteins such as intraflagellar transport 88 (IFT88) and ARL13B were localized to axonemes, indicating transport activity. Taken together, it seems that GBM cells, from both cell lines and tumour tissue, are not generally able to extend primary cilia; this observation is consistent with serum starvation experiments (common protocol to arrest cultured cells in G0 and promote primary cilium assembly) that did not show an enhanced ciliogenesis in GBM cells. However, further experiments are needed to confirm these ideas, especially in patient-derived primary cultures, as cell lines do not precisely reflect human GBM characteristics, and also in larger human cohorts. Interestingly, the downregulation of cilia-related genes in GBM recently reported by Shpak and colleagues based on TCGA gene expression annotations may give support to the decreased ciliation observed in this malignancy.

But apart from descriptive data, what is known about the functional significance of primary cilia in GBM initiation and progression? There is currently little evidence on whether GBM development is cilia-dependent, but several recent works have made substantial progress to determine if primary cilia can affect GBM proliferation. It is necessary to note that cilia can either induce or suppress tumorigenesis, depending on the oncocogenic driver event, and that both roles are likely to take place in GBM. Thus, Yang
and colleagues\textsuperscript{60} established for the first time that primary cilia loss mediated by cyclin-dependent kinase 20 (CDK20; formerly named as cell-cycle related kinase or CCRK) overexpression promotes GBM proliferation in the U-251 MG cell line; this abnormal growth was inhibited when depleting CDK20/CCRK and subsequently ciliogenesis was restored. Notably, this protein was reported to be highly expressed in glioma patients.\textsuperscript{61} This result, together with the reduced expression in animals.\textsuperscript{71,72} It should be noted that, to our knowledge, neither a link between CP55 and primary cilia has been established, nor was the ciliary status of these three astrocytoma/GBM cell lines before and after CP55 depletion evaluated.

Furthermore, it has been proposed that primary cilia could play key roles in common features of GBM such as chemoresistance and tumour invasion. In this sense, while it is true that the functional significance of the ciliated GBM cell subpopulations still remains elusive, new evidence provides exciting avenues for further research. Intriguingly, GBM ciliated cells are known to express zinc finger E-box binding homeobox 1 (ZEB1),\textsuperscript{56} which is a transcription factor recently characterized as the central inducer of the epithelial–mesenchymal transition in GBM, leading to tumorigenesis, invasion and chemoresistance.\textsuperscript{69} In detail, ZEB1 has been proposed as a good candidate for tumour recurrence since it is mainly expressed in invasive GBM cells that also display poor sensitivity to TMZ mediated by MGMT; in addition to regulating the expression of glioma stemness-related factors such as sex determining region Y (SRY)-box 2 (SOX2) and oligodendrocyte transcription factor 2 (OLIG2); these results were confirmed in both primary GBM cell lines and xenograft models.\textsuperscript{69} Remarkably, about 45\% of tumours analysed were ZEB1-positive and that correlated with worse prognosis (shorter survival rates and poorer response to TMZ treatment).\textsuperscript{69} In relation to this, the lack of primary cilium in PCM1-depleted and KIF3A-depleted primary GBM cells has been suggested to underlie the increased sensitivity to TMZ observed in such cells;\textsuperscript{66} therefore, ciliary proteins that mediate ciliogenesis, for example PCM1 and KIF3A, may modulate GBM survival. Moreover, Sarkisian and colleagues\textsuperscript{56} have pointed out that ARL13B, an astrocytic cilia marker also expressed in GBM ciliated cells as stated earlier, might play a guiding role for tumour cells to mediate GBM invasion since interneuronal migration in the developing brain is cilia-dependent and indeed requires ARL13B.\textsuperscript{70} Figure 2 summarizes the main roles of primary cilia in GBM.

On the other hand, it is worth considering the role of the regulatory factor X (RFX) family of transcription factors in GBM since they are well-established, key regulators of ciliary gene expression in animals.\textsuperscript{71,72} Thus, RFX1 is silenced...
in GBM cell lines and tumours due to differential intronic hypermethylation,\textsuperscript{73} and was also shown to inhibit GBM proliferation when overexpressed both \textit{in vitro} and \textit{in vivo}.\textsuperscript{73,74} \textit{RFX1} could therefore be a new tumour suppressor gene in GBM that would further impact cell invasion and survival, since it was demonstrated to inhibit CD44 expression and this led to a decrease in proliferation, invasion and survival of GBM cells.\textsuperscript{74} Interestingly, \textit{RFX1}, \textit{RFX2} and \textit{RFX3} cooperate to modulate the maintenance of neural progenitors and GSCs through regulating fibroblast growth factor 1 (FGF1) promoter activity, an important mitogen for neurogenesis.\textsuperscript{75,76} It would be necessary to explore whether \textit{RFX1} mediates GBM progression through primary cilia, since this possibility was not addressed in the abovementioned studies. In this sense, although the biological role of \textit{RFX1} is not well defined and includes regulation of ciliary and non-ciliary targets,\textsuperscript{72} \textit{RFX1} and \textit{RFX2} were recently demonstrated to regulate the transcription level of \textit{ALMS1} gene, which encodes a protein localized to basal bodies and centrosomes that is mutated in Alström syndrome, a ciliopathy.\textsuperscript{77} It is also worth mentioning that some of the most well-characterized transcription factors in relation to GBM and GSCs such as signal transducer and activator of transcription 3 (STAT3) and several members of the SOX family have been linked to primary cilia; however, a direct regulation by primary cilia in GBM has not yet been reported. With regard to STAT3, it represents a convergence point for most of the oncogenic signalling pathways involved in GBM and is a pivotal
promoter of GSC maintenance, tumour invasion, angiogenesis and immune evasion. Remarkably, mistrafficking of leptin receptor and other receptor complexes in hypothalamic neurons due to primary cilia dysfunction has been found to inactive STAT3 signalling and lead to obesity phenotypes. Moreover, SOX factors are important mediators of tumorigenesis in a number of cancers such as GBM, where SOX2, SOX4 and SOX9 act as oncogenes that play central roles in the maintenance of GSCs to sustain cell stemness and tumorigenicity, and promote tumour proliferation, in addition to being associated with poor clinical outcomes. Intriguingly, combined knockout of Sox4 and Sox9 was reported to inhibit ciliogenesis in cholangiocytes, which could be related to the TGF-β and Notch deregulation observed in the deleted mice analysed.

**Primary cilium as potential mediator of signalling pathways involved in glioblastoma**

The crucial role of primary cilium in mediating virtually all the cellular signalling cascades could anticipate an important but still scarcely explored function in GBM. In fact, deregulation of signalling pathways is a common feature underlying most tumours that decisively contributes to initiating tumorigenesis, as stated above. Moreover, primary cilia responsiveness to changing extracellular conditions could represent a strong adaptive mechanism to modulate tumour progression. It is also of interest to highlight that most of the altered signalling cascades related to GBM are well defined as cilia-regulated pathways. Thus, signal transduction mediated by RTKs such as the epidermal growth factor receptor (EGFR) and especially the platelet-derived growth factor receptor alpha (PDGFRA), as well as Shh, TGF-β, Wnt, and Notch pathways, among others, are known to be coordinated by the primary cilium as sustained by a large body of evidence. It is therefore reasonable to think that primary cilia could be involved in the cellular signalling deregulation observed in many GBM patients. Since EGFR and PDGFRA are key drivers of classical and proneural GBMs, these subtypes might be more affected by cilia-related alterations.

Despite this, there is very little evidence on whether cilia-dependent regulation of signalling cascades is taking place in GBM, as detailed below. On the one hand, receptors of several pathways that significantly contribute to GBM have been localized to the primary cilium of astrocytes, such as PTCH1, EGFR and PDGFRA. In addition, the primary cilium assembled by human astrocytes was reported to have an invagination of the plasma membrane that surrounds the proximal axoneme and contains endocytic vesicles, called the “cilium-pit” [see Figure 2(a)]. Remarkably, this membrane domain at the ciliary base was also detected in primary cilia from GBM tumours in both intact and abnormal form, as well as in GBM primary cell lines. This specialized structure corresponds to the one currently known as the ciliary pocket, which is well-characterized as an active site for exo- and endocytosis of receptors and other ciliary proteins that play a key role in cilia-mediated signalling. In this sense, endocytic control of receptor availability is required for modulating signal transduction in all the pathways listed above, a process that is tightly coupled to the ciliary pocket. Taken together, all these findings represent indirect evidence that primary cilium may actually be contributing to signalling disturbances found in GBM.

Turning now to functional studies, it is surprising that only three studies have so far investigated potential links between cilia and signalling in the pathological context of GBM, as will be described below. Of the three core pathways frequently altered in GBM – retinoblastoma (RB) signalling, tumour protein 53 (p53) signalling and RTK/RAS/PI3K signalling – only the latter has been shown to have some connection with primary cilia in GBM models. Thus, Yang and colleagues reported that the PI3K pathway, which is altered in nearly 90% of GBM, could be related to primary cilia loss via CDK20/CCRK. In this work, U-251 MG cells treated with LY294002, a broad-spectrum inhibitor of PI3Ks, were described to have increased ciliogenesis and reduced CDK20/CCRK mRNA levels. In the same study, the authors had demonstrated that CDK20/CCRK overexpression in U-251 MG cells led to primary cilia loss and subsequent increase in GBM proliferation, which is consistent with those findings after PI3K inhibition. Aberrant activation of the PI3K pathway could thereby impact cilia maintenance in GBM by promoting CDK20/CCRK expression, which would be particularly relevant for the proneural GBM subtype as it was reported to have increased activation of this signalling pathway. There is, however, no current evidence that primary cilium has any role in coordinating...
EGFR and PDGFRA-mediated signalling in GBM, although PDGFRA has long been associated with primary cilia, and EGFR has recently been shown to suppress ciliogenesis in RPE1 cells, involving primary cilia in EGFR-mediated cell proliferation.

On the other hand, Hoang-Minh and colleagues conducted a more comprehensive work focused on the Shh pathway and showed for the first time that some patient-derived GBM cell lines are able to transduce Shh-mediated signalling through primary cilia to promote cell proliferation in vitro. In detail, proliferation of L0 control cells (derived from a 43-year-old male patient) was increased after exogenous stimulation of the Shh pathway, whereas this effect was reversed with a cycloplamine pretreatment (a well-known inhibitor of Shh signalling by blocking SMO entry into cilia) and also by inhibition of ciliogenesis through KIF3A disruption, suggesting that unrestrained growth in GBM is Shh-dependent and mediated by primary cilia. Remarkably, ciliary recruitment of endogenous SMO and GLI family zinc finger 3 (GLI3) was observed following Shh stimulation. Moreover, mice xenografted with L0-KIF3A disrupted cells displayed longer survival rates than those transplanted with L0 control cells. Nevertheless, these effects were highly cell-line specific so that the S3 line (from a 75-year-old male) was completely unaffected by all the assayed experimental conditions, whereas the S2 line (from a 50-year-old male) behaved in the opposite way compared with the L0 cell line, showing non-response to Shh treatment in control cells but decreased cell proliferation after Shh stimulation in cells lacking KIF3A protein. In addition, xenografts with S2-KIF3A disrupted cells showed shorter survival times, possibly due to an increased tumorigenic activity of unmodified S2 cells as supported by the higher baseline proliferation rate observed in the absence of KIF3A/cilia in vitro. Primary cilia could therefore also mediate proliferation restraint in GBM, so it seems that the primary cilium is able to modulate GBM growth by Shh signalling, both in vitro and in vivo, playing a dual role in GBM tumorigenesis. This finding might have greater impact on the classical GBM subtype since it was described to highly express several components of this pathway, although a specific role for Shh signalling in IDH-mutated GBMs, which mostly correspond to the proneural subtype, has also been proposed. However, it is important to emphasize that, in comparison with medulloblastoma, where cilia and Shh pathway play a relevant role, GBM has few Shh or Shh-downstream target-dependent cases.

Finally, a very recent work by Loskutov and colleagues provides highly valuable information about how lysophosphatidic acid (LPA)-mediated signalling can significantly impact GBM proliferation via primary cilium. LPA is a recognized, lipid-based mitogen that is highly enriched in the brain and has a well-established role in GBM, where it mediates proliferation, invasion and angiogenesis properties. Importantly, LPA signals through binding on LPA receptors (LPARs), of which lysophosphatidic acid receptor 1 (LPAR1) is the predominant member in the CNS and is moreover highly expressed in GBM and GSCs. LPARs belong to the family of GPCRs, many of which are well-known to be specifically targeted to cilia, in this sense, endogenous LPAR1 was also recently found to localize to the astrocytic primary cilium. In this study, Loskutov and colleagues demonstrated that loss of primary cilium is enough to stimulate proliferation of human primary astrocytes as well as that this increased growth is LPA-dependent in a cilia-mediated manner. Thus, the authors described a cellular compartmentalization of the LPA signalling machinery in ciliated cells, which restricts LPA signalling and prevents unlimited proliferation in unmodified astrocytes, so that LPAR1 is confined to the cilium, whereas its downstream effectors, Go12 and Goq, are restricted to cytoplasm. When primary cilia are lost by IFT88 or KIF3B depletion, this physical barrier disappears and LPAR1 redistributes to the plasma membrane, where it associates with Go12/Goq to transduce LPA-mediated signalling, thereby promoting the unlimited growth of astrocytes and GBM patient-derived cells. Moreover, inhibition of the LPA pathway with Ki16425 (a LPAR1/3 antagonist) dramatically decreased cell growth rate in deciliated highly proliferative astrocytes, GBM patient-derived cells and xenografts. Even more relevant, intracranial injections of Ki16425 loaded into PEG-PLGA nanoparticles showed a great ability to inhibit tumoural growth in GBM patient-derived xenografts, which represents a promising therapeutic strategy.

Concluding remarks
The emerging role of primary cilia in tumorigenesis that has been reported over the last decade is bringing us a plethora of outstanding findings that
are decisively contributing to a more in-depth understanding of cancer pathogenesis and also to development of novel, cilia-targeted therapeutic strategies. Thus, the key role of primary cilium in both cell cycle control and cellular signalling coordination anticipates an important contribution to cancer that is actually evidenced in its involvement in tumour initiation, invasion and migration, chemoresistance and cancer stem cell maintenance for many different tumours such as medulloblastoma or basal cell carcinoma as two relevant examples. Concerning GBM, considered the most aggressive primary brain tumour, several studies have started to address the potential role of this organelle in GBM progression, so the current evidence presented in this review represents a promising background that justifies further research in this field (summarized in Table 1).

Is primary cilia loss a cause or a consequence of malignant transformation in GBM? When and how are primary cilia lost? Why do only certain GBM cell subpopulations remain ciliated? Are specific GBM subtypes more affected by changes in primary cilia? The available reports so far suggest that GBM cells display mechanisms to inhibit ciliogenesis; in fact, although only small percentages of ciliated cells are observed in GBM samples, most clones from patient-derived GBM cell cultures were shown to be able to form cilia. However, nothing is known about if ciliary loss occurs early in GBM tumorigenesis or whether primary cilium formation depends on the stage of differentiation of GBM cells. In this sense, it...
would be especially intriguing to investigate whether primary cilia could be associated with GSCs, as has been shown for mammary tumour-initiating cells.96 This is particularly important as most studies have been developed in glioma conventional cell lines. Does the primary cilium mediate other signalling pathways involved in the pathogenesis of GBM, apart from Shh and LPA signalling? Has the ciliary pocket any role in this regulation? Given the well-established role of primary cilium in coordinating many of the signalling pathways that influence GBM formation and progression, such as PDGF, TGF-β or Notch signalling, a ciliary coordination of more signalling cascades and their crosstalk in GBM is quite likely to exist and should be promptly addressed. In addition, abnormalities in ciliary length and morphology are known to disturb signal transduction mediated by the primary cilium,97–99 so it may be worth comparing cilia lengths between GBM and normal samples and its potential impact on signalling. On this point, a recent work by Jenks and colleagues100 revealed that drug-resistant cancer cells show increased cilia frequency and length, cilia tip fragmentation and also enhanced Shh activation, which undoubtedly reinforces this line of research in GBM. On the other hand, it is tempting to speculate on the following issue: Does the primary cilium release extracellular vesicles in the context of GBM? Have these vesicles any bioactive role in GBM initiation and progression? Over the last years, an emerging body of evidence points out that primary cilia are able to release extracellular vesicles to maintain ciliary composition, modulate signal transduction and contribute to cell-to-cell communication in different physiological and pathological conditions.101,102 In turn, extracellular vesicles are emerging as key mediators within the tumour microenvironment to promote tumour growth and metastasis for many cancers,103 including GBM, where they have been involved in tumour initiation, proliferation, invasiveness and chemoresistance.104–108 A potential connection between extracellular vesicles and cilia in GBM may therefore be worthy of further investigation.

Taken together, all the evidence presented here reinforces the potential role of the primary cilium as a critical mediator of GBM tumorigenesis and progression, which opens the way to the application of cilia-targeted therapies (‘ciliotherapy’) as a new approach in the fight against this devastating tumour. In this sense, restoration of primary cilia is an increasing strategy to restrain tumour growth in cancer,109,110 and also for GBM; therefore, further strategies based on targeting specific ciliary proteins and/or modulating cilia-dependent signalling in the context of GBM are expected to gain momentum in a few years. This ‘ciliary approach’ to GBM treatment could be especially relevant considering that clinical trials with targeted therapies, especially involving RTK targeting, have failed to improve patient outcome.15 Thus, treatment failures resulting from the extensive GBM intratumoral heterogeneity, with a mixture of cells that show different RTK coactivation throughout time and space,15 might be overcome if targeting a structural component such as the primary cilium. The recent report that manipulating ciliary length/integrity in different drug-resistant cancer cell lines re-sensitizes them to appropriate RTK inhibitors could represent a promising strategy to explore in the context of GBM.108 Finally, it might be interesting to consider strategies based on inhibiting histone deacetylase 6 (HDAC6) to restore primary cilium in GBM, since HDAC6 activation and ciliary loss seem to be common events in a variety of cancers38 and HDAC inhibitors are emerging as a promising group of epigenetic agents for GBM treatment.108,109 We expect that the outbreak of primary cilium as a key mediator of GBM will bring exciting new findings over the coming years that positively impact patient survival.

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References
1. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol 2016; 131: 803–820.
2. Ostrom QT, Bauchet L, Davis FG, et al. The epidemiology of glioma in adults: a “state of the science” review. Neuro Oncol 2014; 16: 896–913.
3. Thakkar JP, Dolecek TA, Horbinski C, et al. Epidemiologic and molecular prognostic review of glioblastoma. *Cancer Epidemiol Biomarkers Prev* 2014; 23: 1985–1996.

4. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005; 352: 987–996.

5. Ohgaki H and Kleihues P. The definition of primary and secondary glioblastoma. *Clin Cancer Res* 2013; 19: 764–772.

6. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008; 455: 1061–1068.

7. Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. *Cell* 2013; 155: 462–477.

8. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 2010; 17: 98–110.

9. Noushmehr H, Weisenberger DJ, Diefes K, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell* 2010; 17: 98–110.

10. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med* 2000; 343: 1350–1354.

11. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005; 352: 997–1003.

12. Sottoriva A, Spiteri I, Piccirillo SGM, et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. *Proc Natl Acad Sci U S A* 2013; 110: 4009–4014.

13. Patel AP, Tirosch I, Trombetta JJ, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science* 2014; 344: 1396–1401.

14. Furnari FB, Cloughesy TF, Cavenee WK, et al. Heterogeneity of epidermal growth factor receptor signaling networks in glioblastoma. *Nat Rev Cancer* 2015; 15: 302–310.

15. Qazi MA, Vora P, Venugopal C, et al. Intratumoral heterogeneity: pathways to treatment resistance and relapse in human glioblastoma. *Ann Oncol* 2017; 28: 1448–1456.

16. Galli R, Binda E, Orfaneli U, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res* 2004; 64: 7011–7021.

17. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. *Nature* 2004; 432: 396–401.

18. Lathia JD, Mack SC, Mulkearns-Hubert EE, et al. Cancer stem cells in glioblastoma. *Genes Dev* 2015; 29: 1203–1217.

19. Ishikawa H and Marshall WF. Ciliogenesis: building the cell’s antenna. *Nat Rev Mol Cell Biol* 2011; 12: 222–234.

20. Elliott KH and Brugmann SA. Sending mixed signals: cilia-dependent signaling during development and disease. *Dev Biol* Epub ahead of print 13 March 2018. DOI: 10.1016/j.ydbio.2018.03.007.

21. Nachury MV, Seeley ES and Jin H. Trafficking to the ciliary membrane: how to get across the periciliary diffusion barrier? *Annu Rev Cell Dev Biol* 2010; 26: 59–87.

22. Garcia-Gonzalo FR and Reiter JF. Open sesame: how transition fibers and the transition zone control ciliary composition. *Cold Spring Harb Perspect Biol* 2017; 9: a028134.

23. Pedersen LB, Veland IR, Schroder JM, et al. Assembly of primary cilia. *Dev Dyn* 2008; 237: 1993–2006.

24. Vertii A, Hehnly H and Doxsey S. The centrosome, a multitalented renaissance organelle. *Cold Spring Harb Perspect Biol* 2017; 9: a028167.

25. Wheway G, Nazlamova L and Hancock JT. Signaling through the primary cilium. *Front Cell Dev Biol* 2018; 6: 8.

26. Han YG and Alvarez-Buylla A. Role of primary cilia in brain development and cancer. *Curr Opin Neurobiol* 2010; 20: 58–67.
30. Youn YH and Han YG. Primary cilia in brain development and diseases. *Am J Pathol* 2018; 188: 11–22.

31. Green JA and Mykytyn K. Neuronal ciliary signaling in homeostasis and disease. *Cell Mol Life Sci* 2010; 67: 3287–3297.

32. Mitchison HM and Valente EM. Motile and non-motile cilia in human pathology: from function to phenotypes. *J Pathol* 2017; 241: 294–309.

33. Reiter JF and Leroux MR. Genes and molecular pathways underpinning ciliopathies. *Nat Rev Mol Cell Biol* 2017; 18: 533–547.

34. Valente EM, Rosti RO, Gibbs E, et al. Primary cilia in neurodevelopmental disorders. *Nat Rev Neurol* 2014; 10: 27–36.

35. Vaisse C, Reiter JF and Berbari NF. Cilia and obesity. *Cold Spring Harb Perspect Biol* 2017; 9: a028217.

36. Hassounah NB, Bunch TA and McDermott KM. Molecular pathways: the role of primary cilia in cancer progression and therapeutics with a focus on Hedgehog signaling. *Clin Cancer Res* 2012; 18: 2429–2435.

37. Seeger-Nukpezah T, Little JL, Serzhanova V, et al. Cilia and cilia-associated proteins in cancer. *Drug Discov Today Dis Mech* 2013; 10: e135–e142.

38. Gradilone SA, Pisarello MJL and LaRusso NF. Primary cilia in tumor biology: the primary cilium as a therapeutic target in cholangiocarcinoma. *Curr Drug Targets* 2017; 18: 958–963.

39. Izawa I, Goto H, Kasahara K, et al. Current topics of functional links between primary cilia and cell cycle. *Cilia* 2015; 4: 12.

40. Briscoe J and Thérond PP. The mechanisms of Hedgehog signaling and its roles in development and disease. *Nat Rev Mol Cell Biol* 2013; 14: 416–429.

41. Bangs F and Anderson KV. Primary cilia and mammalian Hedgehog signaling. *Cold Spring Harb Perspect Biol* 2017; 9: a028175.

42. Pampliega O, Orhon I, Patel B, et al. Functional interaction between autophagy and ciliogenesis. *Nature* 2013; 502: 194–200.

43. Cao M and Zhong Q. Cilia in autophagy and cancer. *Cilia* 2016; 5: 4.

44. Berbari NF, Bishop GA, Askwith CC, et al. Hippocampal neurons possess primary cilia in culture. *J Neurosci Res* 2007; 85: 1095–1100.

45. Bishop GA, Berbari NF, Lewis J, et al. Type III adenyl cyclase localizes to primary cilia throughout the adult mouse brain. *J Comp Neurol* 2007; 505: 562–571.

46. Yoshimura K, Kawate T and Takeda S. Signaling through the primary cilium affects glial cell survival under a stressed environment. *Glia* 2011; 59: 333–344.

47. Kasahara K, Miyoshi K, Murakami S, et al. Visualization of astrocytic primary cilia in the mouse brain by immunofluorescent analysis using the cilia marker Arl13b. *Acta Med Okayama* 2014; 68: 317–322.

48. Sipos É, Komoly S and Ács P. Quantitative comparison of primary cilium marker expression and length in the mouse brain. *J Mol Neurosci* 2018; 64: 397–409.

49. Zhang Q, Hu J and Ling K. Molecular views of Arf-like small GTPases in cilia and ciliopathies. *Exp Cell Res* 2013; 319: 2316–2322.

50. Breunig JJ, Sarkisian MR, Arellano JI, et al. Primary cilium regulate hippocampal neurogenesis by mediating sonic hedgehog signaling. *Proc Natl Acad Sci U S A* 2008; 105: 13127–13132.

51. Han YG, Spassky N, Romaguera-Ros M, et al. Hedgehog signaling and primary cilia are required for the formation of adult neural stem cells. *Nat Neurosci* 2008; 11: 277–284.

52. Tong CK, Han YG, Shah JK, et al. Primary cilia are required in a unique subpopulation of neural progenitors. *Proc Natl Acad Sci U S A* 2014; 111: 12438–12443.

53. Tani E and Ametani T. Ciliated human astrocytoma cells. *Acta Neuropathol* 1970; 15: 208–219.

54. Moser JJ, Fritzler MJ and Rattner JB. Primary ciliogenesis defects are associated with human astrocytoma/glioblastoma cells. *BMC Cancer* 2009; 9: 448.

55. Moser JJ, Fritzler MJ and Rattner JB. Ultrastructural characterization of primary cilia in pathologically characterized human glioblastoma multiforme (GBM) tumors. *BMC Clin Pathol* 2014; 14: 40.

56. Sarkisian MR, Siebzehrubl D, Hoang-Minh L, et al. Detection of primary cilia in human glioblastoma. *J Neurooncol* 2014; 117: 15–24.

57. Shpak M, Goldberg MM and Cowperthwaite MC. Cilia gene expression patterns in cancer. *Cancer Genomics Proteomics* 2014; 11: 13–24.

58. Han YG, Kim HJ, Dlugosz AA, et al. Dual and opposing roles of primary cilia in medulloblastoma development. *Nat Med* 2009; 15: 1062–1065.
59. Wong SY, Seol AD, So PL, et al. Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. *Nat Med* 2009; 15: 1055–1061.

60. Yang Y, Roine N and Mäkelä TP. CCRK depletion inhibits glioblastoma cell proliferation in a cilium-dependent manner. *EMBO Rep* 2013; 14: 741–747.

61. Ng SS, Cheung YT, An XM, et al. Cell cycle-related kinase: a novel candidate oncogene in human glioblastoma. *J Natl Cancer Inst* 2007; 99: 936–948.

62. Loskutov YV, Griffin CL, Marinak KM, et al. LPA signaling is regulated through the primary cilium: a novel target in glioblastoma. *Oncogene* 2018; 37: 1457–1471.

63. Seeley ES, Carrière C, Goetze T, et al. Pancreatic cancer and precursor pancreatic intraepithelial neoplasia lesions are devoid of primary cilia. *Cancer Res* 2009; 69: 422–430.

64. Barakat MT, Humke EW and Scott MP. Kif3a is necessary for initiation and maintenance of medulloblastoma. *Carcinogenesis* 2013; 34: 1382–1392.

65. Hoang-Minh LB, Deleyrolle LP, Siebzehnrubl D, et al. Disruption of KIF3A in patient-derived glioblastoma cells: effects on ciliogenesis, hedgehog sensitivity, and tumorigenesis. *Oncotarget* 2016; 7: 7029–7043.

66. Hoang-Minh LB, Deleyrolle LP, Nakamura NS, et al. PCM1 depletion inhibits glioblastoma cell ciliogenesis and increases cell death and sensitivity to temozolomide. *Transl Oncol* 2016; 9: 392–402.

67. Wang G, Liu M, Wang H, et al. Centrosomal protein of 55 regulates glucose metabolism, proliferation and apoptosis of glioma cells via the Akt/mTOR signaling pathway. *J Cancer* 2016; 7: 1431–1440.

68. Zhu H, Chen D, Tang J, et al. Overexpression of centrosomal protein 55 regulates the proliferation of glioma cell and mediates proliferation promoted by EGFR/III in glioblastoma U251 cells. *Oncol Lett* 2018; 15: 2700–2706.

69. Siebzehnrubl FA, Silver DJ, Tugertimur B, et al. The ZEB1 pathway links glioblastoma initiation, invasion and chemoresistance. *EMBO Mol Med* 2013; 5: 1196–1212.

70. Higginbotham H, Eom TY, Mariani LE, et al. Arl13b in primary cilia regulates the migration and placement of interneurons in the developing cerebral cortex. *Dev Cell* 2012; 23: 925–938.

71. Piasecki BP, Burghoorn J and Swoboda P. Regulatory factor X (RFX)-mediated transcriptional rewiring of ciliary genes in animals. *Proc Natl Acad Sci U S A* 2010; 107: 12969–12974.

72. Choksi SP, Lauter G, Swoboda P, et al. Switching on cilia: transcriptional networks regulating ciliogenesis. *Development* 2014; 141: 1427–1441.

73. Ohashi Y, Ueda M, Kawase T, et al. Identification of an epigenetically silenced gene, RFX1, in human glioma cells using restriction landmark genomic scanning. *Oncogene* 2004; 23: 7772–7779.

74. Feng C, Zhang Y, Yin J, et al. Regulatory factor X1 is a new tumor suppressive transcription factor that acts via direct downregulation of CD44 in glioblastoma. *Neuro Oncol* 2014; 16: 1078–1085.

75. Hsu YC, Liao WC, Kao CY, et al. Regulation of FGF1 gene promoter through transcription factor RFX1. *J Biol Chem* 2010; 285: 13885–13895.

76. Hsu YC, Kao CY, Chung YF, et al. Ciliogenic RFX transcription factors regulate FGF1 gene promoter. *J Cell Biochem* 2012; 113: 2511–2522.

77. Purvis TL, Hearn T, Spalluto C, et al. Transcriptional regulation of the Alström syndrome gene ALMS1 by members of the RFX family and Sp1. *Gene* 2010; 460: 20–29.

78. Chang N, Ahn SH, Kong DS, et al. The role of STAT3 in glioblastoma progression through dual influences on tumor cells and the immune microenvironment. *Mol Cell Endocrinol* 2017; 451: 53–65.

79. Oh EC, Vasanth S and Katsanis N. Metabolic regulation and energy homeostasis through the primary cilium. *Cell Metab* 2015; 21: 21–31.

80. De la Rocha AM, Sampron N, Alonso MM, et al. Role of SOX family of transcription factors in central nervous system tumors. *Am J Cancer Res* 2014; 4: 312–324.

81. Poncy A, Antoniou A, Cordi S, et al. Transcription factors SOX4 and SOX9 cooperatively control development of bile ducts. *Dev Biol* 2015; 404: 136–148.

82. Christensen ST, Clement CA, Satir P, et al. Primary cilia and coordination of receptor tyrosine kinase (RTK) signaling. *J Pathol* 2012; 226: 172–184.

83. Wallingford JB and Mitchell B. Strange as it may seem: the many links between Wnt
signaling, planar cell polarity, and cilia. Genes Dev 2011; 25: 201–213.

84. Clement CA, Ajbro KD, Kofod K, et al. TGF-β signaling is associated with endocytosis at the pocket region of the primary cilium. Cell Rep 2013; 3: 1806–1814.

85. Ezratty EJ, Stokes N, Chai S, et al. A role for the primary cilium in Notch signaling and epidermal differentiation during skin development. Cell 2011; 145: 1129–1141.

86. Leitch CC, Lodh S, Prieto-Echagüe V, et al. Basal body proteins regulate Notch signaling through endosomal trafficking. J Cell Sci 2014; 127(Pt 11): 2407–2419.

87. Danilov AI, Gomes-Leal W, Ahlenius H, et al. Ultrastructural and antigenic properties of neural stem cells and their progeny in adult rat subventricular zone. Glia 2009; 57: 136–152.

88. Benmerah A. The ciliary pocket. Curr Opin Cell Biol 2013; 25: 78–84.

89. Pedersen LB, Mogensen JB and Christensen ST. Endocytic control of cellular signaling at the primary cilium. Trends Biochem Sci 2016; 41: 784–797.

90. Sorkin A and von Zastrow M. Endocytosis and signaling: intertwining molecular networks. Nat Rev Mol Cell Biol 2009; 10: 609–622.

91. Kasahara K, Aoki H, Kiyono T, et al. EGFR receptor kinase suppresses ciliogenesis through activation of USP8 deubiquitinase. Nat Commun 2018; 9: 758.

92. Valadez JG, Grover VK, Carter MD, et al. Identification of Hedgehog pathway responsive glioblastomas by isocitrate dehydrogenase mutation. Cancer Lett 2013; 328: 297–306.

93. Tabuchi S. The autotaxin–lysophosphatidic acid–lysophosphatidic acid receptor cascade: proposal of a novel potential therapeutic target for treating glioblastoma multiforme. Lipids Health Dis 2015; 14: 56.

94. Schou KB, Pedersen LB and Christensen ST. Ins and outs of GPCR signaling in primary cilia. EMBO Rep 2015; 16: 1099–1113.

95. Mykytyn K and Askwith C. G-protein-coupled receptor signaling in cilia. Cold Spring Harb Perspect Biol 2017; 9: a028183.

96. Guen VJ, Chavarria TE, Kröger C, et al. EMT programs promote basal mammary stem cell and tumor-initiating cell stemness by inducing primary ciliogenesis and Hedgehog signaling. Proc Natl Acad Sci U S A 2017; 114: E10532–E10539.

97. Ocbina PJR, Eggenschwiler JT, Moskowitz IP, et al. Complex interactions between genes controlling trafficking in primary cilia. Nat Genet 2011; 43: 547–553.

98. Dummer A, Poelma C, DeRuiter MC, et al. Measuring the primary cilium length: improved method for unbiased high-throughput analysis. Cilia 2016; 5: 7.

99. Hilgendorf KI, Johnson CT and Jackson PK. The primary cilium as a cellular receiver: organizing ciliary GPCR signaling. Curr Opin Cell Biol 2016; 39: 84–92.

100. Jenks AD, Vyse S, Wong JP, et al. Primary cilium mediates diverse kinase inhibitor resistance mechanisms in cancer. Cell Rep 2018; 23: 3024–3055.

101. Nager AR, Goldstein JS, Herranz-Pérez V, et al. An actin network dispatches ciliary GPCRs into extracellular vesicles to modulate signaling. Cell 2017; 168: 252–263.e14.

102. Wang J and Barr MM. Ciliary extracellular vesicles: txt msg organelles. Cell Mol Neurobiol 2016; 36: 449–457.

103. Xu R, Rai A, Chen M, et al. Extracellular vesicles in cancer: implications for future improvements in cancer care. Nat Rev Clin Oncol. Epub ahead of print 23 May 2018. DOI: 10.1038/s41571-018-0036-9.

104. Oushy S, Hellwinkel JE, Wang M, et al. Glioblastoma multiforme-derived extracellular vesicles drive normal astrocytes towards a tumour-enhancing phenotype. Philos Trans R Soc Lond B Biol Sci 2018; 373: 20160477.

105. Gourlay J, Morokhoff AP, Luwor RB, et al. The emergent role of exosomes in glioma. J Clin Neurosci 2017; 35: 13–23.

106. Quezada C, Torres Á, Niechi I, et al. Role of extracellular vesicles in glioma progression. Mol Aspects Med 2018; 60: 38–51.

107. Khan NA, Willemarck N, Talebi A, et al. Identification of drugs that restore primary cilium expression in cancer cells. Oncotarget 2016; 7: 9975–9992.

108. Arrizabalaga O, Moreno-Cugnon L, Auzmendi-Iriarte J, et al. High expression of MKP1/DUSP1 counteracts glioma stem cell activity and mediates HDAC inhibitor response. Oncogenesis 2017; 6: 401.

109. Bezczyn P. Histone deacetylase inhibitors in glioblastoma: pre-clinical and clinical experience. Med Oncol 2014; 31: 985.