Cellular Signalling 57 (2019) 2–9

Contents lists available at ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

Review

Understanding and exploiting cell signalling convergence nodes and pathway cross-talk in malignant brain cancer

Nok Him Fung[a,1], Corrina A. Grima[a,1], Samuel S. Widodo[a], Andrew H. Kaye[b,c], Clarissa A. Whitehead[b], Stanley S. Stylli[b,c], Theo Mantamadiotis[a,b,*]

[a] Department of Microbiology and Immunology, The University of Melbourne, Melbourne, VIC 3000, Australia
[b] Department of Surgery, The University of Melbourne, The Royal Melbourne Hospital, Parkville, VIC 3050, Australia
[c] Department of Neurosurgery, The Royal Melbourne Hospital, Parkville, VIC 3050, Australia

ABSTRACT

In cancer, complex intracellular and intercellular signals constantly evolve for the advantage of the tumour cells but to the disadvantage of the whole organism. Decades of intensive research have revealed the critical roles of cellular signalling pathways in regulating complex cell behaviours which influence tumour development, growth and therapeutic response, and ultimately patient outcome. Most studies have focussed on specific pathways and the resulting tumour cell function in a rather linear fashion, partly due to the available methodologies and partly due to the traditionally reductionist approach to research. Advances in cancer research, including genomic technologies have led to a deep appreciation of the complex signals and pathway interactions operating in tumour cells. In this review we examine the role and interaction of three major cell signalling pathways, PI3K, MAPK and cAMP, in regulating tumour cell functions and discuss the prospects for exploiting this knowledge to better treat difficult to treat cancers, using glioblastoma, the most common and deadly malignant brain cancer, as the example disease.

1. Introduction

Glioblastoma (GBM), is an aggressive and invariably lethal form of malignant brain tumour. GBM is highly heterogeneous and invasive, which accounts for the fact that it has one of the worst survival rates across all types of cancers. The highly complex nature of GBM also impacts advances toward the development of better treatments. The best current standard treatment across the globe relies on surgery, radiotherapy and a single drug, temozolomide (TMZ) [1]. The median survival time with optimal treatment is about 14 months, while the 5-year survival rate is close to or below 5% [2]. The uniformly poor prognosis in the majority of GBM patients highlights the importance of research needed to understand the molecular pathways and mechanisms which drive GBM pathology as a means to identify better and safer treatments.

1.1. Primary and secondary GBM

GBM can occur as a primary, de novo tumour, or as a secondary tumour developing from pre-existing lower grade glioma tumours. In primary GBM, tumours develop rapidly, within several months, without evidence of pre-existing symptoms or neoplasms. Secondary GBM arises

Abbreviations: AC, Adenylyl cyclase; AKT, AKT8 virus oncogene cellular homolog; ATP, Adenosine triphosphate; BBB, Blood brain barrier; Bim, BCL2-Like 11 protein; cAMP, Cyclic adenosine 3′,5′-monophosphate; CRAF, C-Raf proto-oncogene; CREB, cAMP Response Element Binding Protein; EGFR, Epidermal growth factor receptor; ERK, Extracellular signal-regulated kinase; GBM, Glioblastoma; GDP, Guanosine diphosphate; GPCR, G-protein-coupled receptor; GRB2, Growth factor receptor bound protein-2; GTP, Guanosine triphosphate; IDH1, Isocitrate dehydrogenase 1; LGG, Low grade glioma; MAPK, Mitogen-activated protein kinase; MAPKKK, Mitogen-activated protein kinase kinase kinase; MEK, MAPK/ERK kinase; MMP, Matrix-metalloproteinase; mTOR, Mammalian target of rapamycin; NF1, Neurofibromin 1; PD-1, Programmed cell death-1 protein; PDE, Phosphodiesterase; PDGFRA, Platelet derived growth factor receptor alpha; PDK, 3-phosphoinositide-dependent protein kinase; PD-L1, Programmed death-ligand 1; PI3K, Phosphoinositide 3-kinase; PKA, Protein kinase A; PTEN, Phosphatase and tensin homolog; RAF, Rapidly accelerated fibrosarcoma; RAPTOR, Regulatory-associated protein of mtor; RHEB, Ras homolog enriched in brain; RSK, Ribosomal s6 kinase; RTK, Receptor tyrosine kinase; SOS, Son of sevenless homolog; TCGA, The Cancer Genome Atlas; TMZ, Temozolomide; TORC, Target of rapamycin complex; TP53, Tumour protein 53; TSC, Tuberous sclerosis complex

* Corresponding author at: Department of Microbiology and Immunology, The University of Melbourne, Melbourne, VIC 3000, Australia.
E-mail address: theom@unimelb.edu.au (T. Mantamadiotis).

[1] Equal contribution.

https://doi.org/10.1016/j.cellsig.2019.01.011
Received 17 January 2019; Received in revised form 29 January 2019; Accepted 29 January 2019
Available online 30 January 2019
0898-6568/

from a lower grade tumour. For example, diffuse astrocytoma, a grade II tumour, can gradually progress into malignant anaplastic astrocytoma (grade III), and ultimately into GBM (grade IV). Progression from low grade glioma (LGG) to GBM is slow, occurring over the course of several years, which is reflected in the longer median survival time of LGG patients compared to GBM patients [3].

2. Genomics and GBM biology

2.1. GBM subtypes

In a landmark study by The Cancer Genome Atlas (TCGA) consortium involving the consolidation of clinical data together with DNA sequencing, expression analysis via microarray and RNA sequencing of hundreds of patient tumours, four GBM molecular subtypes have been identified which, in part, explain some variations in clinical the characteristics observed and patient outcomes [4].

2.1.1. Classical GBM

The classical subtype of GBM can be identified by the amplification of the epidermal growth factor receptor (*EGFR*) gene, as well as point mutations or variant III (vIII) mutations within the *EGFR* gene [5]. Tumour protein 53 (TP53) is not typically mutated in the classical subtype, despite being frequently altered in the other subtypes. Patients with classical GBM also respond best to aggressive treatment, possibly because of the lack of TP53 mutations in tumour cells [4].

2.1.2. Mesenchymal GBM

Tumours in the mesenchymal subtype are associated with a higher rate of neurofibromin 1 (NF1) and phosphatase and tensin homolog (PTEN) mutations, which are regulatory components of the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K) pathways respectively. TP53 mutations are also common in mesenchymal GBM. Like classical GBM, aggressive treatment significantly improves the overall survival of GBM patients with this subtype [4].

2.1.3. Proneural GBM

The proneural subtype correlates with younger patients and is characterized by focal amplification on chromosome 4q12, within which the platelet derived growth factor receptor alpha (PDGFRA) gene is located [6]. Although this amplification is also seen in other subtypes, only in proneural GBM is it accompanied by high levels of PDGFRA expression. In addition, isocitrate dehydrogenase 1 (IDH1) and TP53 mutations are also closely associated with proneural GBM. Interestingly, these features are also characteristic of many secondary GBM, which are also enriched for the proneural subtype. Patients with proneural GBM have a longer median survival time, are younger but are less responsive to aggressive treatment [4].

2.1.4. Neural GBM

Unlike other subtypes, there is no obvious pattern of gene amplification or mutation in the neural subtype. Rather, neural GBMs are characterized by their expression of typical neuronal genes. Patients with neural GBM have the worst survival rate of all subtypes, and this is only marginally improved with aggressive treatment [4].

2.2. Tumour cell invasion in GBM

GBMs are highly proliferative and invasive, making complete surgical resection of tumours virtually impossible. Thus, even with adjuvant radiotherapy and chemotherapy after surgery, almost all cases of GBM recur within 6–9 months of initial treatment, often within 1–2 cm of the original tumour [7].

In GBM the expression of matrix-metalloproteinases (MMPs) 2 and 9 are upregulated [8]. Up-regulation of these matrix-metalloproteinases are the result of several deregulated signalling pathways in GBM, including the phosphoinositide 3-kinase (PI3K) pathway [9]. Such upregulation of MMPs is thought to contribute greatly to the invasiveness of GBM as it allows GBM cells to degrade the extracellular matrix and invade neighbouring tissues via structures known as invadopodia [10,11]. Furthermore, PI3K is activated via several extracellular signals, such as growth factor receptor signalling, and shown to promote invadopodia formation [12], while PI3K inhibitors such as LY294002, inhibit invadopodia formation [13].

2.3. Cellular heterogeneity in GBM

The original full name for GBM, glioblastoma multiforme, describes the heterogeneous histopathological appearance of these tumours. Advances in gene technology has extended observations of the morphological heterogeneity of GBM, to include the genetic heterogeneity that underlies the histopathology. Improved imaging and antibody biomarkers has also led to a more sophisticated understanding of the cellular composition of GBM and an appreciation of the role and contribution of non-tumour cells, including endothelial cells and immune cells, to disease progression, treatment resistance and recurrence [14,15].

3. Drug resistance

GBM is relatively resistant to therapy, with recurrent GBM being even more resistant, even to highly aggressive treatment and is the result of a complex interplay of factors. A primary reason for poor efficacy of many anticancer drugs which are used for other cancers, is inefficient drug penetrance due to the impediment of the Blood Brain Barrier (BBB) and high tumour interstitial pressure [16,17]. This is compounded by genomic instability induced by widespread alterations in genetic material which results in extensive genotypic and phenotypic heterogeneity producing clonal populations of cells which are resistant to any single course of therapy, including TMZ [18,19]). GBM is also an extremely invasive cancer, rapidly spreading to neighbouring structures and extending through into healthy brain tissue [20]. Invading cells extend past the visible tumour mass margin, escaping surgical resection and radiation therapy and the cells serve as a source of tumour recurrence. The tumour mass also harbours rare cancer stem-like cells and it is this population of cells which exhibit robust radio-resistance, which may contribute to cellular heterogeneity [19,21].

4. Multiple signal transduction pathways promote GBM carcinogenesis

4.1. MAPK signalling

The activity of the mitogen activated protein kinase (MAPK) pathway is frequently altered in GBM. This pathway has key roles regulating cell proliferation, cell survival and metastasis (J.-Y. [22,23]). High levels of phosphorylated (activated) MAPK has been linked to poor patient survival in GBM [24].

The MAPK pathway can be activated when growth factor ligands such as epidermal growth factor (EGF) bind to their corresponding receptors, which belong to the receptor tyrosine kinase (RTK) family (Fig. 1). Upon ligand binding the RTK undergoes dimerization and autophosphorylation which triggers a cascade of phosphorylation events via various protein substrates. RTK autophosphorylation leads to the recruitment of adaptor proteins, such as growth factor receptor bound protein-2 (GRB2), and Son of sevenless homolog (SOS) [25]. SOS promotes the removal of guanosine diphosphate (GDP) from Ras and recruitment of guanosine triphosphate (GTP) [26]. This conversion activates Ras which binds and activates the kinase RAF at the membrane which then activates MEK (also known as MAPK kinase) which in turn activates MAPK. This pathway is negatively regulated by the

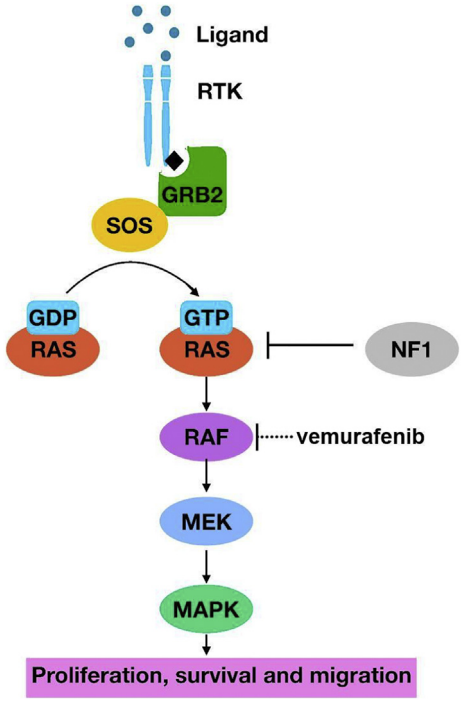

Fig. 1. The MAPK Pathway. The pathway is activated by a ligand binding to its corresponding receptor tyrosine kinase (RTK), resulting in the receptor dimerization and phosphorylation. This recruits adaptor proteins which activate RAS. This begins a signalling cascade in which Ras activates RAF, which in turn activates MEK which then activates MAPK (ERK1/2). MAPK has a number of downstream effects such as proliferation, survival and migration. NF1 negatively regulates this pathway by converting GTP to GDP, inactivating RAS. Vemurafenib is a drug which inhibits RAF (BRAF), as indicated by the dotted line.

tumour suppressor NF1 via the hydrolysis of GTP, converting it to GDP and inactivating Ras [27].

In cancer, multiple components of this signalling cascade may been transformed resulting in hyperactivation of this pathway, which dives tumour malignancy. The epidermal growth factor receptor (*EGFR*) gene is the most commonly amplified gene in GBM [28], seen in approximately 40% of GBM which drives the hyper-activation of both the MAPK and phosphoinositide 3-kinase (PI3K) pathways. The negative regulator NF1 is deleted or mutationally inactivated in 14% of GBM cases, according to The Cancer Genome Atlas [29]. This aberration is most often seen in mesenchymal GBM, with occurrences in up to 37% of cases. The activation of one of the terminal MAPK substrates, such as ERK (extracellular signal-regulated kinase) or p38, leads to nuclear its translocation where it exerts a number of effects via activation of several transcription factors. These transcription factors include the proto-oncogenes, Elk1, c-myc, Ets and cAMP Response Element Binding Protein (CREB) [30–32].

4.2. PI3K signalling

The PI3K pathway is one of the most frequently perturbed pathways in cancer and regulates multiple cellular functions including cell differentiation, adhesion, motility, invasion, proliferation and survival [33,34]. Similar to the MAPK pathway, PI3K signalling occurs via multiple steps and is a tightly regulated process (Fig. 2). Activation can occur following growth factor stimulation of receptor tyrosine kinases (RTKs) resulting in activation of PI3K via interaction of the catalytic p110 and p85 regulatory subunits and conversion of phosphatidylinositol (4, 5)-bisphosphate (PIP_2) lipids to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). PKB/Akt binds to PIP_3 at the plasma membrane,

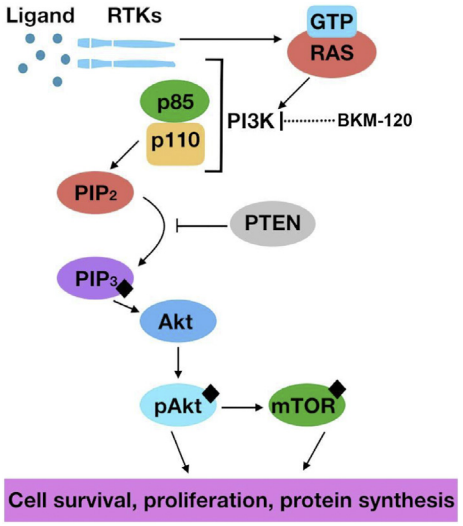

Fig. 2. The PI3K Pathway. This pathway is activated by binding of ligand to a receptor tyrosine kinase (RTK). This causes the dimerization and phosphorylation of the RTK. PI3K is recruited to the RTK via the regulatory subunit p85 and activates the catalytic subunit, p110. PI3K can also be activated by active members of the Ras family. The p110 catalytic subunit converts PIP2 to PIP3, a process which is negatively regulated by PTEN. PIP3 causes AKT to undergo phosphorylation and activation. AKT signals to a number of downstream factors including mTOR, to promote cell survival, proliferation and protein synthesis. BKM120 is a drug which inhibits PI3K, as indicated by the dotted line.

triggering PDK1 to phosphorylate and activate Akt [35]. PI3K can also be activated by members of the Ras family [36]. Akt has several downstream targets, one of which is mTOR. In the homeostatic or non-cancer state, mTOR monitors cellular conditions to regulate cell growth and proliferation [37] and upon hyperactivation of the PI3K pathway, mTOR is also hyperactivated and acts on multiple targets which promote protein synthesis. A key negative regulatory mechanism of the PI3K pathway involves the actions of the phosphatase and tensin homolog (PTEN) protein, which dephosphorylates PIP_3 to suppress signalling [38]. Multiple components of the PI3K pathway may be mutated or amplified in GBM, including mutation and/or amplification the EGFR [28], activating mutations or amplification of *PIK3CA*, the gene encoding the p110 catalytic subunit of PI3K or inactivating mutations or deletion of tumour suppressor PTEN [5].

4.3. Crosstalk and convergence between the PI3K and MAPK pathways

Apart from self-regulation, the PI3K and MAPK pathways also cross-regulate each other at multiple levels at many points along their signalling cascades. Importantly, both pathways can be activated by the same cell surface receptors, including RTKs and GPCRs. RAS, which typically involved in MAPK signalling, also activates PI3K [36] (Fig. 4). Moreover, the GAB1 protein which can bind to the GRB2-SOS complex is also an activator of PI3K [39]. Like AKT, ERK can inhibit the downstream PI3K factor, TSC1/2, leading to the upregulation of RHEB-GTP, and subsequent activation of mTORC1 [40]. In addition, ERK can also directly promote mTORC1 by phosphorylating the mTORC1 component regulatory-associated protein of mTOR (RAPTOR) [41] and/or ribosomal s6 kinase (RSK) [42].

Apart from cross-activation, ERK also possesses cross-inhibitory functions. ERK can downregulate the PI3K/AKT pathway by phosphorylating and inhibiting GAB1, which reduces activation of PI3K [39]. On the other hand, the PI3K/AKT pathway can also downregulate the MAPK pathway through the action of AKT which can phosphorylate RAF to inhibit it [43].

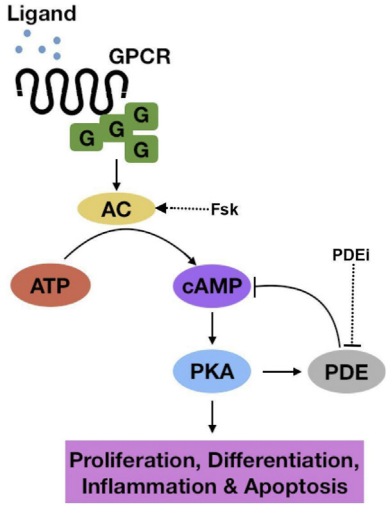

Fig. 3. The cAMP pathway. Upon ligand binding to G-Protein-Coupled Receptors (GPCR), the enzyme Adenylyl Cyclase (AC) converts Adenosine Triphosphate (ATP) to cAMP. cAMP in turn activates Protein Kinase A (PKA) to inhibit tumour biology. The enzyme phosphodiesterase (PDE) negatively regulates this pathway. The pathway can be activated to promote apoptosis via drug agonists such as forskolin/Fsk or phosphodiesterase inhibitors (PDEi), as indicated by the dotted lines.

5. cAMP signalling

The cyclic adenosine 3′, 5′-monophosphate (cAMP) pathway regulates multiple cellular functions. Signalling typically begins with the binding of a ligand to a G-protein-coupled receptor (GPCR) [44]. This causes G-proteins to activate the enzyme adenylyl cyclase (AC) which converts adenosine triphosphate (ATP) to the second messenger cAMP (Fig. 3). Subsequently, cAMP can activate several effectors including the cAMP dependent protein kinase A (PKA). An important regulatory mechanism of this pathway is that occurs via negative regulation by phosphodiesterases (PDEs), which degrade cAMP [45]. The cAMP pathway interacts with other pathways, including MAPK and PI3K. For example, cAMP activated PKA can inhibit the MAPKKK, Raf [46]. However, PKA has also be shown to inhibit the ERK tyrosine phosphatase, PTP, increasing ERK signalling [47]. Likewise, the cAMP pathway can interact with the PI3K pathway, exemplified by the effect of follicle stimulating hormone, FSH, in ovarian cells [48].

Compared to MAPK and PI3K signalling, research into the role of the cAMP pathway in tumours has been less prominent but long-studied. This is perhaps due to observations that cAMP activation in tumours is generally low, unlike the increased activation of MAPK and PI3K signalling. Furman and Shulman's work, published in 1977, investigated the levels of cAMP and AC in brain tumour samples compared to normal brain tissue and found a significant reduction in both cAMP and AC levels in the tumours samples [49]. Notably, GBM and other higher

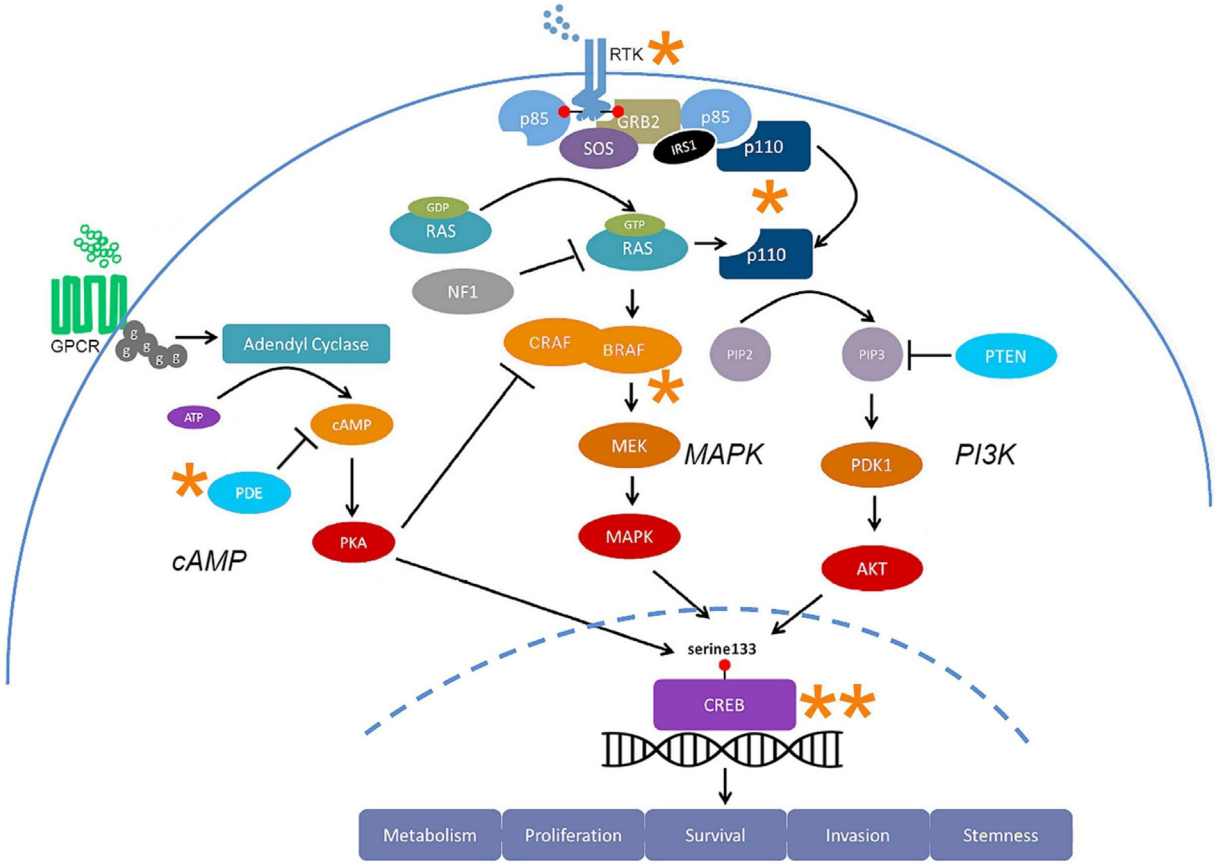

Fig. 4. Convergence of PI3K, MAPK and cAMP pathways on CREB. Signals are initiated by ligand binding to specific receptors, either G-Protein-Coupled Receptors (GPCR) or receptor tyrosine kinases (RTK). CREB sits at a hub downstream of the pathways and the terminal kinases from each pathway phosphorylate CREB, at amino acid Ser133, which leads to recruitment of transcriptional *co*-activators and the RNA polymerase complex, to activate the expression of target genes. The precise spectrum of target genes expressed will differ depending on level of the upstream signal and cooperation with other transcription factors and will regulate many cellular functions including metabolism, proliferation, survival, invasion and stemness. There are several pathway interaction points indicated where cross-talk/cross-regulation (activation or inhibition) can occur. Asterisks indicate key positions in the pathways where drug targeted inhibition can be achieved, noting that at transcription factor hubs, where signals converge onto CREB in this example, would potentially overcome bypass due to drug-induced adaptation at a single upstream node, as indicated by the double asterisk.

tumour grade glioma samples had some of the lowest cAMP levels, compared to lower grade tumours. While mutations in this pathway are not common in cancer, there is evidence that altered expression of AC and PDE may be responsible for decreased levels of cAMP in tumours [50]. The inverse relationship between cAMP levels and tumour malignancy and a view that cAMP signalling is tumour suppressive, has more recently generate renewed interest in this pathway in cancer biology and the possibility the pathway is a potential therapeutic target in cancers such as GBM. Supporting this view, the elevation of cAMP levels via inhibition of PDEs has been shown to inhibit growth, increase differentiation and promote apoptosis in GBM cells [50,51]. Similarly, upon administering a PDE inhibitor in an orthotopic xenograft mouse model of brain cancer, tumour growth rate was inhibited [52]. Moreover, the chemical nature of some PDE inhibitors, such as diazepam (Valium), and the reason for their use and FDA-approval for neurological disorders means these drugs should efficiently cross the BBB [53]; a key drug property for effective GBM treatment. Recent research suggests that PDE4 inhibitors may be tailored to use for brain cancer therapy (reviewed in [53,54]).

5.1. Integration of multiple signalling pathways in the nucleus

Multiple signalling pathways converge on transcriptional hubs, which function to coordinate upstream signals and regulate and fine-tune cellular responses. The cAMP response element-binding protein (CREB) is a transcription factor with numerous downstream functions, including neurogenesis and cancer [55]. CREB activation via phosphorylation of a key serine residue (Ser133) is regulated by several cell signalling pathways including the cAMP, PI3K and MAPK pathways (Fig. 4) [55,56]. CREB is important for normal brain development and its deletion in mouse brain leads to neurodegeneration in the developing brain, and hippocampal and striatal neurodegeneration in postnatal brain [57]. Other in vivo models show that CREB is required for efficient neurogenesis and that CREB overexpression results in neural stem/progenitor cell hyper-proliferation [58,59]. Elevated CREB expression, CREB deletion or inhibition modulates tumour growth in various experimental models [60–62]). Moreover, CREB expression and activation correlates with patient survival in various malignant diseases [63,64].

6. Targeting signal transduction pathways in brain cancer

6.1. Targeting the PI3K and MAPK pathways

The PI3K and MAPK pathways regulate GBM development and tumour growth but the precise cellular behaviours regulated by each pathway is not fully understood. Given the limited treatment strategies currently available to patients, the development of new drugs which target the PI3K and MAPK pathways could potentially improve overall patient survival and quality of life significantly. In fact, numerous preclinical studies and clinical trials have been undertaken using inhibitors of the PI3K and MAPK pathways in GBM.

One of the most promising drugs is the potent pan-PI3K inhibitor, BKM120 (Buparlisib). It targets the PI3K heterodimer and therefore downregulates all downstream effectors of the PI3K pathway [65] (Fig. 2). It is currently in multiple phase I/II/III clinical trials for several cancers including breast cancer and GBMs [66,67]. Preclinical studies have shown that BKM-120 suppresses invasiveness of GBM cells in vitro and in vivo [68].

Vemurafenib is a MAPK pathway inhibitor which targets the BRAF protein, downstream of Ras (Fig. 1). It is currently FDA approved for the treatment of late-stage melanoma [69], and is also approved for metastatic melanoma patients with the $BRAF^{V600E}$ mutation [70]. Several clinical studies have also shown great promise for the use of vemurafenib in GBM treatment. A case study using vemurafenib reported complete regression of a recurrent paediatric GBM with a $BRAF^{V600E}$ mutation [71], with further clinical studies providing evidence of selective efficacy in subsets of GBM patients [72]).

Drugs targeting receptor tyrosine kinases (RTK) or their ligands have also been tested in GBM patients. Erlotinib is an EGFR inhibitor and is FDA-approved for several cancers including non-small cell lung cancer [73]. A phase II trial combining erlotinib and bevacizumab with standard first line therapy for newly diagnosed GBM patients found that, although patients experienced longer progression-free survival, overall survival was not significantly increased [66]. In a remarkable, although a single case report, a patient with recurrent GBM has survived for eight years while undergoing therapy using a combination of bevacizumab and erlotinib [74].

6.2. Co-targeting the PI3K/MAPK Pathways in GBM

Inhibitors that target specific components of the PI3K and MAPK pathways are in clinical use, but the success of these has been limited by the drug resistance that develops over time. Studies have shown that tumour cells respond to chronic drug treatment by adapting their signalling circuitry, taking advantage of pathway redundancy, to maintain their growth and function. For example, in colorectal cancer, activation of ErbB2 signalling can bypass the block, induced by EGFR antibody inhibitors [75]. Using specific combinations of drugs will enforce multiple signalling blocks that cannot be circumvented, enhancing the prospects of tumour cell growth inhibition or tumour cell death. Two independent studies examined the effects of using a combination of PI3K and MAPK drugs in glioma models to show that drug resistance was suppressed and malignancy reduced [76,77].

6.3. Targeting pathway convergence and crosstalk nodes

An alternative approach to minimize or delay the development of treatment resistance by alternate pathway activation, could be to target downstream signalling hubs. Considering the pro-oncogenic signals transmitted via the MAPK and PI3K pathways, targeting the CREB transcription factor on which these pathways converge may be an effective approach. By targeting specific pathway nodes and signalling hub transcription factors, such as CREB, the problem of alternate pro-oncogenic pathway activation would also be minimized, compared to inhibition of only one pathway. Moreover, toxicity may be minimized by using a single agent which inhibits multiple pathways, rather than a combination of drugs targeting each pathway individually, to achieve similar biochemical effect. Recent research showed that two structurally-related small molecule CREB inhibitor drugs were able to inhibit leukemia cells in vivo and small cell lung cancer cells, in vitro [78,79], raising the possibility of targeting CREB in cancer patients, for more effective tumour cell inhibition.

6.4. Targeting the cAMP pathway

The close association of cAMP with other oncogenic cell signalling pathways and the association between low cAMP levels and tumour malignancy makes this pathway a promising target for therapeutics. A recent study showed that activation of the cAMP pathway using the AC activator, forskolin (FSK) and the PDE inhibitor, isobutylmethylxanthine (IBMX) induced cell death in A172 and T98G GBM cell lines, via Bim-regulated apoptosis [50]. Interestingly, in the same study, other GBM cell lines exhibited significant resistance to cAMP-induced cell death at the same drug concentrations. It turns out that the resistant cells exhibited relatively high MAPK activation and that when a combination of MAPK inhibitor and cAMP activation was used, Bim expression increased and apoptosis-mediated cell death was triggered.

As the cAMP pathway normally activates CREB, there appear to be opposing pro- and anti-oncogenic functions activated, when also considering PI3K- and MAPK-dependent CREB activation. Although, the

Table 1
Examples of targeted combination therapies and immunotherapies used in recent or current clinical trials for glioblastoma. mAb: monoclonal antibody.

Therapy Mode	Drug	Clinical trial/pre-clinical model	Trial phase	National Clinical Trial ID/ Reference
Combined angiogenic + PI3K inhibitors	Bevacizumab (anti-VEGF mab) + bkm120 (PI3K inhibitor)	Clinical trial	II	NCT01349660
Combined angiogenic + EGFR inhibitors	Bevacizumab (anti-VEGF) + erlotinib (anti-EGFR) mabs	Clinical trial	II	NCT005255525
Combined angiogenic inhibitor + immunotherapy	Bevacizumab (anti-VEGF) + pembrolizumab (anti-PD-L1) mabs	Clinical trial	II	NCT02337491
Combined MAPK + PI3K inhibitors	MAPK inhibitors: trametinib, selumetinib PI3K inhibitors: buparlisib, dactolisib	Pre-clinical mouse models: genetically engineered mice & patient-derived xenografts	–	[77]
Combined MAPK + PI3K inhibitors	MAPK inhibitor: trametinib PI3K inhibitor: everolimus	Preclinical mouse model: cell xenografts	–	[76]

precise CREB-dependent signals activated in GBM cells via cAMP are not fully understood, our studies show that cAMP-induced Bim expression and apoptosis, does not involve CREB [50]. Rather, a complex interaction between the cAMP and MAPK pathways exists, where cAMP activation may inhibit the MAPK pathway via PKA inhibition of CRAF [80].

7. The influence of signalling pathways on the GBM microenvironment

Activation of MAPK and PI3K pathways are linked to immunosuppression in GBM. Upregulation of pERK1/2 in the MAPK pathway and inactivation of PTEN in the PI3K pathway are associated with increased expression of PD-L1 on the surface of GBM cells [81,82]. PD-L1 is an immune checkpoint which can induce T-cell apoptosis when interacting with PD-1 on the T-cell surface. GBM tumours harbouring PTEN inactivating mutations are known to upregulate PD-L1 expression in monocytes through IL-10 modulation [83]. In melanoma, inhibiting PI3K and MAPK pathway can reverse this effect, although the reversal is more effective via MAPK inhibition [84]. Furthermore, combination therapy using MAPK or PI3K inhibitors with PD-L1 inhibitor shows great promise in mouse cancer models [85,86]. Although similar studies have not been done in GBM, combination targeted therapy and immunotherapy may be key to more effective treatments for GBM patients. Although tumour cells require multiple signalling pathways to function, in GBM tissue there is evidence that there is regional and specific activation of the PI3K and MAPK pathways, suggesting that although these pathways can cooperate, they can also function independently [87]. In the same study, analysis of GBM mRNA expression data further suggests that both pathways can activate specific CREB target genes to promote specific cellular functions. Overall, this suggests that modulation of several signal transduction pathways provides dynamic cooperative and pathway-specific functions in GBM cells, which allow the tumour cells to quickly respond to their microenvironment, including development of resistance to exogenous therapeutic challenge.

8. Conclusions

Backed by the evidence from a vast array of research so far, it seems possible that specific targeting of three key cellular signal transduction pathways may lead to an improved clinical management of difficult to treat cancers, such as GBM. Targeting individual pathways in GBM is unlikely to provide effective tumour control, but using a combinatorial drug approach, each targeting distinct, pivotal positions or nodes in multiple pathways may lead to safer treatment and improved patient outcome. Experimental evidence suggests that a combinatorial drug-based treatment will target critical cancer cell functions, killing cells and minimising drug resistance. Indeed, as GBM exhibits heterogeneous activation of the PI3K and MAPK pathways [87]), at the very least, both of these pathways need to be targeted. To further maximise the impact on GBM cell viability, activation of the suppressed cAMP pathway could potentially trigger apoptotic mechanisms to enhance GBM control. Of course, there are a number of critical issues which need to be carefully determined when drug combinations are to be used in patients. Most importantly, toxicity of drug combinations needs to be determined in the context of the minimal tumour effective drug concentration. The effectiveness of targeted anti-cancer drugs also needs to be determined in the context of standard brain cancer therapeutic modalities, including TMZ chemotherapy and radiotherapy in GBM. Moreover, BM, will likely require additional treatments to provide long-term improvement in patient outcome, including the use of novel immunotherapies. Evidence of the innovative multimodal approach to GBM treatment is seen through the establishment of recent clinical trials using drugs which target multiple kinases and signalling nodes in the MAPK and PI3K pathways, as well as testing the efficacy of novel immunotherapeutic approaches, in combination with the pathway inhibitors (Table 1).

Acknowledgements

Our laboratories are supported by grants from the CASS Foundation Australia, The Brain Foundation Australia and the Royal Melbourne Hospital Neuroscience Foundation.

References

[1] Roger Stupp, Warren P. Mason, Martin J. van den Bent, Michael Weller, Barbara Fisher, Martin J.B. Taphoorn, Karl Belanger, et al., Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma, N. Engl. J. Med. 352 (10) (2005) 987–996.
[2] P.D. Delgado-López, E.M. Corrales-García, Survival in glioblastoma: a review on the impact of treatment modalities, Clin. Transl. Oncol. 18 (11) (2016) 1062–1071.
[3] Hiroko Ohgaki, Paul Kleihues, Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas, J. Neuropathol. Exp. Neurol. 64 (6) (2005) 479–489.
[4] Roel G.W. Verhaak, Katherine A. Hoadley, Elizabeth Purdom, Victoria Wang, Yuan Qi, Matthew D. Wilkerson, C. Ryan Miller, et al., Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1, Cancer Cell 17 (1) (2010) 98–110.
[5] Beth K. Neilsen, Richard Sleightholm, Rodney McComb, Shakti H. Ramkissoon, Jeffrey S. Ross, Robert J. Corona, Vincent A. Miller, Matthew Cooke, Michele R. Aizenberg, Comprehensive genetic alteration profiling in primary and recurrent glioblastoma, J. Neuro-Oncol. (2018), https://doi.org/10.1007/s11060-018-03070-2.
[6] Heikki Joensuu, Marjut Puputti, Harri Sihto, Olli Tynninen, Nina N. Nupponen, Amplification of genes encoding KIT, PDGFRalpha and VEGFR2 receptor tyrosine kinases is frequent in glioblastoma multiforme, J. Pathol. 207 (2) (2005) 224–231.
[7] Lewis C. Hou, Anand Veeravagu, Andrew R. Hsu, Victor C.K. Tse, Recurrent glioblastoma multiforme: a review of natural history and management options,

Neurosurg. Focus. 20 (4) (2006) E5.
[8] D. Koul, R. Parthasarathy, R. Shen, M.A. Davies, S.A. Jasser, S.K. Chintala, J.S. Rao, et al., Suppression of matrix metalloproteinase-2 gene expression and invasion in human glioma cells by MMAC/PTEN, Oncogene 20 (46) (2001) 6669–6678.
[9] Xing-Chen Chen, Xiang-Tai Wei, Jun-Hong Guan, Hong Shu, Duo Chen, EGF stimulates glioblastoma metastasis by induction of matrix metalloproteinase-9 in an EGFR-dependent mechanism, Oncotarget 8 (39) (2017) 65969–65982.
[10] Stanley S. Stylli, Andrew H. Kaye, Peter Lock, Invadopodia: at the cutting edge of tumour invasion, J. Clin. Neurosci. 15 (7) (2008) 725–737.
[11] Leon Mao, Clarissa A. Whitehead, Lucia Paradiso, Andrew H. Kaye, Andrew P. Morokoff, Rodney B. Luwor, Stanley S. Stylli, Enhancement of invadopodia activity in glioma cells by sublethal doses of irradiation and temozolomide, J. Neurosurg. 129 (3) (2018) 598–610.
[12] Daisuke Hoshino, Kevin M. Branch, Alissa M. Weaver, Signaling inputs to invadopodia and podosomes, J. Cell Sci. 126 (Pt 14) (2013) 2979–2989.
[13] Kelly Harper, Roxane R. Lavoie, Martine Charbonneau, Karine Brochu-Gaudreau, Claire M. Dubois, The hypoxic tumor microenvironment promotes invadopodia formation and metastasis through LPA1 receptor and EGFR cooperation, Mol. Cancer Res. (2018), https://doi.org/10.1158/1541-7786.MCR-17-0649.
[14] Yasuharu Akasaki, Gentao Liu, Nancy H.C. Chung, Moneeb Ehtesham, Keith L. Black, S. Yu John, Induction of a CD4+ T regulatory type 1 response by cyclooxygenase-2-overexpressing glioma, J. Immunol. 173 (7) (2004) 4352–4359.
[15] Charles K. Brown, Nikolai N. Khodarev, Jianqing Yu, Tricia Moo-Young, Edwardine Labay, Thomas E. Darga, Mitchell C. Posner, Ralph R. Weichselbaum, Helena J. Mauceri, Glioblastoma cells block radiation-induced programmed cell death of endothelial cells, FEBS Lett. 565 (1–3) (2004) 167–170.
[16] Y. Boucher, H. Salehi, B. Witwer, G.R. Harsh 4th, R.K. Jain, Interstitial fluid pressure in intracranial tumours in patients and in rodents, Br. J. Cancer 75 (6) (1997) 829–836.
[17] D.R. Groothuis, P. Molnar, R.G. Blasberg, Regional Blood Flow and Blood-to-Tissue Transport in Five Brain Tumor Models, (1984), pp. 132–153.
[18] Mona Meyer, Jüri Reimand, Xiaoyang Lan, Renee Head, Xueming Zhu, Michelle Kushida, Jane Bayani, et al., Single cell-derived clonal analysis of human glioblastoma links functional and genomic heterogeneity, Proc. Natl. Acad. Sci. U. S. A. 112 (3) (2015) 851–856.
[19] Daniel V. Brown, Gulay Filiz, Paul M. Daniel, Frédéric Hollande, Sebastian Dworkin, Stephanie Amiridis, Nicole Kountouri, Wayne Ng, Andrew P. Morokoff, Theo Mantamadiotis, Expression of CD133 and CD44 in glioblastoma stem cells correlates with cell proliferation, phenotype stability and intra-tumor heterogeneity, PLoS One 12 (2) (2017) e0172791.
[20] K. Onda, R. Tanaka, H. Takahashi, N. Takeda, F. Ikuta, Cerebral glioblastoma with cerebrospinal fluid dissemination: a clinicopathological study of 14 cases examined by complete autopsy, Neurosurgery 25 (4) (1989) 533–540.
[21] Sheila K. Singh, Ian D. Clarke, Mizuhiko Terasaki, Victoria E. Bonn, Cynthia Hawkins, Jeremy Squire, Peter B. Dirks, Identification of a cancer stem cell in human brain tumors, Cancer Res. 63 (18) (2003) 5821–5828.
[22] Wen-Ta Chiu, Shing-Chuan Shen, Jyh-Ming Chow, Cheng-Wei Lin, Ling-Tin Shia, Yen-Chou Chen, Contribution of reactive oxygen species to migration/invasion of human glioblastoma cells U87 via ERK-dependent COX-2/PGE(2) activation, Neurobiol. Dis. 37 (1) (2010) 118–129.
[23] Jee-Youn Kim, Yong-Jun Kim, Sun Lee, Jae-Hoon Park, The critical role of ERK in death resistance and invasiveness of hypoxia-selected glioblastoma cells, BMC Cancer 9 (2009) 27.
[24] Christian Mawrin, Sabine Diete, Tim Treuheit, Siegfried Kropf, Christian K. Vorwerk, Carsten Boltze, Elmar Kirches, Raimund Firsching, Knut Dietzmann, Prognostic relevance of MAPK expression in glioblastoma multiforme, Int. J. Oncol. 23 (3) (2003) 641–648.
[25] Y. Hu, D.D. Bowtell, Sos1 rapidly associates with Grb2 and is hypophosphorylated when complexed with the EGF receptor after EGF stimulation, Oncogene 12 (9) (1996) 1865–1872.
[26] Shuichi Okada, Jeffrey E. Pessin, Interactions between Src homology (SH) 2/SH3 adapter proteins and the guanylnucleotide exchange factor SOS are differentially regulated by insulin and epidermal growth factor, J. Biol. Chem. 271 (41) (1996) 25533–25538.
[27] N. al-Alawi, G. Xu, R. White, R. Clark, F. McCormick, J.R. Feramisco, Differential regulation of cellular activities by GTPase-activating protein and NF1, Mol. Cell. Biol. 13 (4) (1993) 2497–2503.
[28] Yutaka Hayashi, Keisuke Ueki, Andreas Waha, Otmar D. Wiestler, David N. Louis, Andreas Deimlina, Association of EGFR gene amplification and CDKN2 (p16/MTS1) gene deletion in glioblastoma multiforme, Brain Pathol. 7 (3) (1997) 871–875.
[29] Cancer Genome Atlas Research Network, Comprehensive genomic characterization defines human glioblastoma genes and core pathways, Nature 455 (7216) (2008) 1061–1068.
[30] Sabrina Davis, Peter Vanhoutte, Christiane Pagès, Jocelyne Caboche, Serge Laroche, The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo, J. Neurosci. 20 (12) (2000) 4563–4572.
[31] Jun Xing, David D. Ginty, Michael E. Greenberg, Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase, Science 273 (5277) (1996) 959–963.
[32] C.F. Chuang, S.Y. Ng, Functional divergence of the MAP kinase pathway. ERK1 and ERK2 activate specific transcription factors, FEBS Lett. 346 (2–3) (1994) 229–234.
[33] Susanne Kleber, Ignacio Sancho-Martinez, Benedict Wiestler, Alexandra Beisel, Christian Gieffers, Oliver Hill, Meinolf Thiemann, et al., Yes and PI3K Bind CD95 to signal invasion of glioblastoma, Cancer Cell 13 (3) (2008) 235–248.
[34] M. Osaki, M. Oshimura, H. Ito, PI3K-Akt pathway: its functions and alterations in human cancer, Apoptosis 9 (6) (2004) 667–676.
[35] Dario R. Alessi, Stephen R. James, C. Peter Downes, Andrew B. Holmes, Piers R.J. Gaffney, Colin B. Reese, Philip Cohen, Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bα, Curr. Biol. 7 (4) (1997) 261–269.
[36] Andrea Kauffmann-Zeh, Pablo Rodriguez-Viciana, Eugen Ulrich, Christopher Gilbert, Paul Coffer, Julian Downward, Gerard Evan, Suppression of c-Myc-induced apoptosis by Ras signalling through PI (3) K and PKB, Nature 385 (6616) (1997) 544.
[37] Vander Haar, Seong-Il Lee Emilie, Sricharan Bandhakavi, Timothy J. Griffin, Do-Hyung Kim, Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40, Nat. Cell Biol. 9 (3) (2007) 316–323.
[38] T. Maehama, J.E. Dixon, The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate, J. Biol. Chem. 273 (22) (1998) 13375–13378.
[39] Cheng Fang Yu, Zhen-Xiang Liu, Lloyd G. Cantley, ERK negatively regulates the epidermal growth factor-mediated interaction of Gab1 and the phosphatidylinositol 3-kinase, J. Biol. Chem. 277 (22) (2002) 19382–19388.
[40] Louis-Andre Julien, Audrey Carriere, Julie Moreau, Philippe P. Roux, mTORC1-activated S6K1 phosphorylates rictor on threonine 1135 and regulates mTORC2 signaling, Mol. Cell. Biol. 30 (4) (2010) 908–921.
[41] Audrey Carriere, Yves Romeo, Hugo A. Acosta-Jaquez, Julie Moreau, Eric Bonneil, Pierre Thibault, Diane C. Fingar, Philippe P. Roux, ERK1/2 phosphorylate raptor to promote ras-dependent activation of mTOR complex 1 (mTORC1), J. Biol. Chem. 286 (1) (2011) 567–577.
[42] Bruno D. Fonseca, Tommy Alain, Leona K. Finestone, Brandon P.H. Huang, Mark Rolfe, Tian Jiang, Zhong Yao, Greco Hernandez, Christopher F. Bennett, Christopher G. Proud, Pharmacological and genetic evaluation of proposed roles of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK), extracellular signal-regulated kinase (ERK), and p90(RSK) in the control of mTORC1 protein signaling by phorbol esters, J. Biol. Chem. 286 (31) (2011) 27111–27122.
[43] C. Rommel, B.A. Clarke, S. Zimmermann, L. Nuñez, R. Rossman, K. Reid, K. Moelling, G.D. Yancopoulos, D.J. Glass, Differentiation stage-specific inhibition of the Raf-MEK-ERK pathway by Akt, Science 286 (5445) (1999) 1738–1741.
[44] Paolo Sassone-Corsi, The Cyclic AMP pathway, Cold Spring Harb. Perspect. Biol. 4 (12) (2012), https://doi.org/10.1101/cshperspect.a011148.
[45] R.W. Butcher, E.W. Sutherland, Adenosine 3′,5'-phosphate in biological materials. I. Purification and properties of cyclic 3′,5'-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3′,5'-phosphate in human urine, J. Biol. Chem. 237 (1962) 1244–1250.
[46] M.D. Houslay, W. Kolch, Cell-Type specific integration of cross-talk between extracellular signal-regulated kinase and cAMP signaling, Mol. Pharmacol. 58 (4) (2000) 659–668.
[47] C. Blanco-Aparicio, J. Torres, R. Pulido, A novel regulatory mechanism of MAP kinases activation and nuclear translocation mediated by PKA and the PTP-SL tyrosine phosphatase, J. Cell Biol. 147 (6) (1999) 1129–1136.
[48] Yun-Ju Chen, Pei-Wen Hsiao, Ming-Ting Lee, J. Ian Mason, Ferng-Chun Ke, Jiuan-Jiuan Hwang, Interplay of PI3K and cAMP/PKA signaling, and rapamycin-hypersensitivity in TGFbeta1 enhancement of FSH-stimulated steroidogenesis in rat ovarian granulosa cells, J. Endocrinol. 192 (2) (2007) 405–419.
[49] M.A. Furman, K. Shulman, Cyclic AMP and adenyl cyclase in brain tumors, J. Neurosurg. 46 (4) (1977) 477–483.
[50] Paul M. Daniel, Gulay Filiz, Theo Mantamadiotis, Sensitivity of GBM cells to cAMP agonist-mediated apoptosis correlates with CD44 expression and agonist resistance with MAPK signaling, Cell Death Dis. 7 (12) (2016) e2494.
[51] Thomas C. Chen, Pia Wadsten, Su Susan, Neal Rawlinson, Florence M. Hofman, Colin K. Hill, Axel H. Schonthal, The type IV phosphodiesterase inhibitor rolipram induces expression inhibitors p21Cip1 and p27Kip1, resulting in growth inhibition, increased differentiation, and subsequent apoptosis of malignant A-172 glioma cells, Cancer Biol. Ther. 1 (3) (2002) 268–276.
[52] Lihua Yang, Erin Jackson, B. Mark Woerner, Arie Perry, David Piwnica-Worms, Joshua B. Rubin, Blocking CXCR4-mediated cyclic AMP suppression inhibits brain tumor growth in vivo, Cancer Res. 67 (2) (2007) 651–658.
[53] M.C. Collado, J. Beleta, E. Martinez, M. Miralpeix, T. Domènech, J.M. Palacios, J. Hernández, Functional and biochemical evidence for diazepam as a cyclic nucleotide phosphodiesterase type 4 inhibitor, Br. J. Pharmacol. 123 (6) (1998) 1047–1054.
[54] Rajarshi Sengupta, Tao Sun, Nicole M. Warrington, Joshua B. Rubin, Treating brain tumors with PDE4 Inhibitors, Trends Pharmacol. Sci. 32 (6) (2011) 337–344.
[55] Theo Mantamadiotis, Nikos Papalexis, Sebastian Dworkin, CREB signalling in neural stem/progenitor cells: recent developments and the implications for brain tumour biology, BioEssays 34 (4) (2012) 293–300.
[56] B. Mayr, M. Montminy, Transcriptional regulation by the phosphorylation-dependent factor CREB, Nat. Rev. Mol. Cell Biol. 2 (8) (2001) 599–609.
[57] Theo Mantamadiotis, Thomas Lemberger, Susanne C. Bleckmann, Heidrun Kern, Oliver Kretz, Ana Martin Villalba, François Tronche, et al., Disruption of CREB function in brain leads to neurodegeneration, Nat. Genet. 31 (1) (2002) 47–54.
[58] Sebastian Dworkin, Jordane Malaterre, Frédéric Hollande, Phillip K. Darcy, Robert G. Ramsay, Theo Mantamadiotis, cAMP response element binding protein is required for mouse neural progenitor cell survival and expansion, Stem Cells 27 (6) (2009) 1347–1357.
[59] Sebastian Dworkin, Joan K. Heath, Tanya A. deJong-Curtain, Benjamin M. Hogan, Graham J. Lieschke, Jordane Malaterre, Robert G. Ramsay, Theo Mantamadiotis, CREB activity modulates neural cell proliferation, midbrain-hindbrain organization and patterning in zebrafish, Dev. Biol. 307 (1) (2007) 127–141.

[60] C. Tregnago, E. Manara, M. Zampini, V. Bisio, C. Borga, S. Bresolin, S. Aveic, G. Germano, G. Basso, M. Pigazzi, CREB engages C/EBPδ to initiate leukemogenesis, Leukemia 30 (9) (2016) 1887–1896.
[61] Markus Linder, Elisabeth Glitzner, Sriram Srivatsa, Latifa Bakiri, Kazuhiko Matsuoka, Parastoo Shahrouzi, Monika Dumanic, et al., EGFR is required for FOS-dependent bone tumor development via RSK2/CREB signaling, EMBO Mol. Med. 10 (11) (2018), https://doi.org/10.15252/emmm.201809408.
[62] Yifeng Xia, Cheng Zhan, Mingxiang Feng, Mathias Leblanc, Eugene Ke, Narayana Yeddula, Inder M. Verma, Targeting CREB pathway suppresses small cell lung cancer, Mol. Cancer Res. 16 (5) (2018) 825–832.
[63] Valeria Barresi, Giovanni Branca, Maria Caffo, Giovanni Tuccari, P-CREB expression in human meningiomas: correlation with angiogenesis and recurrence risk, J. Neuro-Oncol. 122 (1) (2015) 87–95.
[64] Deepa B. Shankar, Jerry C. Cheng, Kentaro Kinjo, Noah Federman, Theodore B. Moore, Amandip Gill, Nagesh P. Rao, Elliot M. Landaw, Kathleen M. Sakamoto, The role of CREB as a proto-oncogene in hematopoiesis and in acute myeloid leukemia, Cancer Cell 7 (4) (2005) 351–362.
[65] Matthew T. Burger, Sabina Pecchi, Allan Wagman, Zhi-Jie Ni, Mark Knapp, Thomas Hendrickson, Gordana Atallah, et al., Identification of NVP-BKM120 as a potent, selective, orally bioavailable class I PI3 kinase inhibitor for treating cancer, ACS Med. Chem. Lett. 2 (10) (2011) 774–779.
[66] Jennifer L. Clarke, Annette M. Molinaro, Joanna J. Phillips, Nicholas A. Butowski, Susan M. Chang, Arie Perry, Joseph F. Costello, Ashley A. DeSilva, Jane E. Rabbitt, Michael D. Prados, A single-institution phase II trial of radiation, temozolomide, erlotinib, and bevacizumab for initial treatment of glioblastoma, Neuro-Oncology 16 (7) (2014) 984–990.
[67] Christophe Massard, Kim Nguyen Chi, Daniel Castellano, Johann de Bono, Gwenaelle Gravis, Luc Dirix, Jean-Pascal Machiels, et al., Phase Ib dose-finding study of abiraterone acetate plus buparlisib (BKM120) or dactolisib (BEZ235) in patients with castration-resistant prostate cancer, Eur. J. Cancer 76 (2017) 36–44.
[68] Maria-Carmela Speranza, Michal O. Nowicki, Prajna Behera, Choi-Fong Cho, E. Antonio Chiocca, Sean E. Lawler, BKM-120 (Buparlisib): a phosphatidyl-inositol-3 kinase inhibitor with anti-invasive properties in glioblastoma, Sci. Rep. 6 (2016) 20189.
[69] Ruth Halaban, Wengeng Zhang, Antonella Bacchiocchi, Elaine Cheng, Fabio Parisi, Stephan Ariyan, Michael Krauthammer, James P. McCusker, Yuval Kluger, Mario Sznol, PLX4032, a selective BRAF(V600E) kinase inhibitor, activates the ERK pathway and enhances cell migration and proliferation of BRAF melanoma cells, Pigment Cell Melanoma Res. 23 (2) (2010) 190–200.
[70] Geoffrey Kim, Amy E. McKee, Yang-Min Ning, Maitreyee Hazarika, Marc Theoret, John R. Johnson, Xu Qiang Casey, et al., FDA approval summary: vemurafenib for treatment of unresectable or metastatic melanoma with the BRAFV600E mutation, Clin. Cancer Res. 20 (19) (2014) 4994–5000.
[71] Giles W. Robinson, Brent A. Orr, Amar Gajjar, Complete clinical regression of a BRAF V600E-mutant pediatric glioblastoma multiforme after BRAF inhibitor therapy, BMC Cancer 14 (2014) 258.
[72] Thomas Kaley, Mehdi Touat, Vivek Subbiah, Antoine Hollebecque, Jordi Rodon, A. Craig Lockhart, Vicki Keedy, et al., BRAF inhibition in BRAFV600-mutant gliomas: results from the VE-BASKET study, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. (2018) 3477–3484 (JCO2018789990).
[73] Sean Khozin, Gideon M. Blumenthal, Xiaoping Jiang, Kun He, Karen Boyd, Anthony Murgo, Robert Justice, Patricia Keegan, Richard Pazdur, U.S. Food and Drug Administration approval summary: erlotinib for the first-line treatment of metastatic non-small cell lung cancer with epidermal growth factor receptor Exon 19 deletions or exon 21 (L858R) substitution mutations, Oncologist 19 (7) (2014) 774–779.
[74] Quintino Giorgio D'Alessandris, Nicola Montano, Maurizio Martini, Tonia Cenci, Liverana Lauretti, Vittorio Stumpo, Fabrizio Pignotti, et al., Eight-year survival of a recurrent glioblastoma patient treated with molecularly tailored therapy: a case report, Acta Neurochir. 160 (12) (2018) 2387–2391.
[75] Kimio Yonesaka, Kreshnik Zejnullahu, Isamu Okamoto, Taroh Satoh, Federico Cappuzzo, John Souglakos, Dalia Ercan, et al., Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab, Sci. Transl. Med. 3 (99) (2011) 99ra86.
[76] Payal Jain, Amanda Silva, Harry J. Han, Shih-Shan Lang, Yuankun Zhu, Katie Boucher, Tiffany E. Smith, et al., Overcoming resistance to single-agent therapy for oncogenic BRAF gene fusions via combinatorial targeting of MAPK and PI3K/mTOR signaling pathways, Oncotarget 8 (49) (2017) 84697–84713.
[77] Robert S. McNeill, Demitra A. Canoutas, Timothy J. Stuhlmiller, Harshil D. Dhruv, David M. Irvin, Ryan E. Bash, Steven P. Angus, et al., Combination therapy with potent PI3K and MAPK inhibitors overcomes adaptive kinome resistance to single agents in preclinical models of glioblastoma, Neuro-Oncology 19 (11) (2017) 1469–1480.
[78] Jong Woo Lee, Hee Sun Park, Sin-Aye Park, Seung-Hee Ryu, Wuyi Meng, Juliane M. Jürgensmeier, Jonathan M. Kurie, et al., A novel small-molecule inhibitor targeting CREB-CBP complex possesses anti-cancer effects along with cell cycle regulation, autophagy suppression and endoplasmic reticulum stress, PLoS One 10 (4) (2015) e0122628.
[79] B. Mitton, H.-D. Chae, K. Hsu, R. Dutta, G. Aldana-Masangkay, R. Ferrari, K. Davis, et al., Small molecule inhibition of cAMP response element binding protein in human acute myeloid leukemia cells, Leukemia 30 (12) (2016) 2302–2311.
[80] S. Häfner, H.S. Adler, H. Mischak, P. Janosch, G. Heidecker, A. Wolfman, S. Pippig, M. Lohse, M. Ueffing, W. Kolch, Mechanism of inhibition of Raf-1 by protein kinase A, Mol. Cell. Biol. 14 (10) (1994) 6696–6703.
[81] Andrew T. Parsa, James S. Waldron, Amith Panner, Courtney A. Crane, Ian F. Parney, Jeffrey J. Barry, Kristine E. Cachola, et al., Loss of tumor suppressor PTEN Function increases B7-H1 expression and immunoresistance in glioma, Nat. Med. 13 (1) (2007) 84–88.
[82] Konrad Gabrusiewicz, Xu Li, Jun Wei, Yuuri Hashimoto, Anantha L. Marisetty, Martina Ott, Fei Wang, et al., Glioblastoma stem cell-derived exosomes induce M2 macrophages and PD-L1 expression on human monocytes, Oncoimmunology 7 (4) (2018) e1412909.
[83] Orin Bloch, Courtney A. Crane, Rajwant Kaur, Michael Safaee, Martin J. Rutkowski, Andrew T. Parsa, Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages, Clin. Cancer Res. 19 (12) (2013) 3165–3175.
[84] Xiaofeng Jiang, Jun Zhou, Anita Giobbie-Hurder, Jennifer Wargo, F. Stephen Hodi, The activation of MAPK in melanoma cells resistant to BRAF inhibition promotes PD-L1 expression that is reversible by MEK and PI3K inhibition, Clin. Cancer Res. 19 (3) (2013) 598–609.
[85] Marcel A. Deken, Jules Gadiot, Ekaterina S. Jordanova, Ruben Lacroix, Melissa van Gool, Paula Kroon, Cristina Pineda, et al., Targeting the MAPK and PI3K pathways in combination with PD1 blockade in melanoma, Oncoimmunology 5 (12) (2016) e1238557.
[86] Peter J.R. Ebert, Jeanne Cheung, Yagai Yang, Erin McNamara, Rebecca Hong, Marina Moskalenko, Stephen E. Gould, et al., MAP kinase inhibition promotes T cell and anti-tumor activity in combination with PD-L1 checkpoint blockade, Immunity 44 (3) (2016) 609–621.
[87] Paul M. Daniel, Gulay Filiz, Martin J. Tymms, Robert G. Ramsay, Andrew H. Kaye, Stanley S. Stylli, Theo Mantamadiotis, Intratumor MAPK and PI3K signaling pathway heterogeneity in glioblastoma tissue correlates with CREB signaling and distinct target gene signatures, Exp. Mol. Pathol. 105 (1) (2018) 23–31.