Published in final edited form as:

Trends Pharmacol Sci. 2011 June ; 32(6): 337–344. doi:10.1016/j.tips.2011.02.015.

Treating brain tumors with PDE4 Inhibitors

Rajarshi Sengupta, Tao Sun, Nicole M Warrington, and Joshua B. Rubin

Department of Pediatrics, Campus Box 8208, 660 South Euclid Ave, Washington University
School of Medicine, St Louis, MO 63110

Abstract

Speculation regarding dysregulation of cyclic adenosine monophosphate (cAMP) metabolism in oncogenesis has existed since the discovery of cAMP more than 50 years ago. Recent data confirms the relevance of disordered cAMP metabolism to the genesis of multiple cancers and suggests that the mechanism may involve altered expression and activity of phosphodiesterases (PDEs). These discoveries coincide with the rapid development and clinical evaluation of PDE inhibitors for non-cancer indications. Thus, the time is ripe to evaluate PDE inhibitors as cancer chemotherapeutics. Herein we highlight recent evidence that abnormal regulation of cAMP levels may be a determinant of brain tumorigenesis and that among the mechanisms of its dysregulation is altered expression of PDE. Recent preclinical and clinical experience with inhibitors of PDE4 indicates that this may be a promising approach to brain tumor therapy.

Keywords

cAMP; phosphodiesterase; brain tumor; clinical trial

Introduction

The 1971 Nobel Prize in Physiology or Medicine was awarded to Earl Sutherland for the discovery of the first second messenger, 3', 5'-cyclic adenosine mono-phosphate (cAMP)¹. In his Nobel address, Sutherland noted that, "defective cyclic AMP formation may be involved in the growth of tumors." In our opinion, Sutherland's prediction is proving correct, and therapeutic manipulation of cyclic nucleotide levels may be applicable to multiple cancer types². In this article, we focus on data that suggest low levels of cAMP play a crucial role in brain tumorigenesis. Furthermore, elevating cAMP by blocking its degradation with PDE4 inhibitors might have significant clinical value in the treatment of brain tumors.

Cyclic AMP as a determinant of brain tumorigenesis

The first specific connection between cAMP levels and brain cancers was reported in 1977 by Furman and Schulman³. They found an inverse relationship between cAMP levels and the degree of malignancy in several types of brain tumors. High cAMP levels were

© 2011 Elsevier Ltd. All rights reserved.

Corresponding Author: Rubin, J.B. (rubin_j@kids.wustl.edu).

Disclosure statement for Authors

The authors have no conflicts of interest to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

associated with more benign tumors, whereas lower levels were correlated with greater malignancy. These findings are consistent with subsequent studies indicating that high concentrations of cAMP inhibit the growth of many cell types, including cells derived from the most common forms of adult and pediatric malignant brain tumors, astrocytomas and medulloblastomas, respectively ⁴⁻⁹.

We recently reported that suppression of cAMP in response to ligation of the Gi-coupled G protein coupled receptor (GPCR) CXCR4 was abnormally sustained and associated with growth in cells derived from medulloblastoma and glioblastoma multiforme (GBM), the most malignant form of astrocytoma ⁵. Sustained depression of cAMP inhibited apoptosis and enhanced tumor cell growth. Moreover, in a genetically-engineered mouse model of the autosomal dominant cancer predisposition syndrome neurofibromatosis 1 (NF1), in which mice develop astrocytomas (gliomas) of their optic nerves (optic pathway glioma (OPG)), brain region-specific differences in expression of the CXCR4 ligand, CXCL12, and cAMP levels coincided with the pattern of tumor formation ⁴. The highest levels of CXCL12 and the lowest cAMP levels were found where tumors most commonly occurred in the optic pathway. In the cortex, where gliomas in *Nf1* mice are rarely found, CXCL12 levels are lower and cAMP levels are significantly higher ⁴. These region-specific differences in CXCL12 levels and tumorigenesis strictly parallel what occurs in humans with NF1 ¹⁰.

Warrington *et al.* altered the pattern of cAMP levels in the brain to test the hypothesis that cAMP dictated the pattern of glioma formation in *Nf1* mice. Foci of decreased cAMP levels were created in the cerebral cortex through stereotactic injection of lentivirus encoding the cAMP-specific phosphodiesterase PDE4A1 ¹¹. PDE4A1 is an isoenzyme belonging to the PDE4 subfamily of cAMP-specific phosphodiesterases (Figure 1) ^{12, 13}. It was specifically chosen for these studies because of the high expression of PDE4 in the brain ^{14, 15}, the association of PDE4 with several central nervous system (CNS) disorders ¹⁶⁻¹⁸, the reported stimulation of model brain tumor growth by PDE4A1 overexpression ¹⁹ and the lack of regulation of PDE4A1 enzymatic activity by extracellular signal-regulated kinases (ERK) or protein kinase A (PKA) ²⁰. This latter detail resulted in a predictable level of hydrolytic activity and cAMP suppression.

Cortical overexpression of PDE4A1 resulted in the formation of ectopic tumors resembling the low-grade OPGs seen in both patients with NF1 and in *Nf1* mice (Figure 2) ¹¹. A catalytically inactive form of PDE4A1 (PDE4A1-H229Q²¹) did not induce tumors, suggesting that cAMP suppression was tumorigenic. Whether other mechanisms of cAMP suppression including increased expression of other cAMP specific PDEs would produce similar effects remains to be determined.

Mechanisms of cAMP dysregulation in brain tumors

Overall, the above data indicate that altered cAMP levels may be critical in the genesis and progression of brain tumors. Cyclic AMP is synthesized by adenylyl cyclases (AC) and degraded by a single superfamily of hydrolases, the PDEs. The mechanism(s) underlying altered cAMP signaling in brain tumors are incompletely understood but could include changes in expression and activity in ACs or PDEs.

ACs in brain tumors

To date, 10 different AC isoforms have been cloned and categorized based on their structural features, regulation by heterotrimeric G proteins, protein kinases, calmodulin and intracellular cations (Table 1). As described above, alterations in AC expression exist in brain tumors, and malignant tumors often possess diminished AC activity compared to benign tumors ^{3, 22}. However, not all malignant tumors display reduced AC activity. One of

four molecular subtypes of medulloblastoma was reported to exhibit increased AC1 expression²³.

The mechanistic bases for altered AC capacity in brain tumors remain undefined. Among the potential mechanisms is increased Gi-coupled GPCR expression. Expression of several chemokine receptors is increased in brain tumors of multiple lineages^{24, 25}. Interestingly, the level of CXCR4 expression has prognostic significance in low and high-grade astrocytomas and is elevated in medulloblastoma^{23, 26–28}. CXCR4 mediates interactions between tumor cells and multiple stromal elements including endothelial cells, microglia and entrapped neurons^{4, 29, 30}. Increased Gi-coupled GPCRs would be anticipated to inhibit AC activity and result in lower levels of cAMP. For CXCR4 this has been demonstrated to be critical to its brain tumor promoting activity⁵.

Degradation of cAMP

Twenty-one mammalian genes encoding cyclic nucleotide PDEs have been identified and are subdivided into 11 PDE subfamilies (PDE1 through PDE11) based on sequence homology, substrate specificity and inhibitor sensitivity. Among these, five PDE subfamilies are capable of hydrolyzing both cAMP and cGMP (PDE 1-3, 10, 11), and three are cAMP-specific (PDE 4, 7, 8.) The remainder, (PDE 5, 6 and 9) are cGMP-specific³¹. Some PDE sub-families, like PDE4, contain multiple genes, each of which encode several isoforms that are generated through alternative splicing and exhibit unique patterns of developmental and tissue specific expression and regulation (Table 2). Of note, intracellular cAMP signaling is highly compartmentalized, primarily through the subcellular localization of PDEs to particular membrane domains and macromolecular complexes through specific localization motifs. The relationship between PDE structure and compartmentalized cAMP signaling has been extensively detailed for the PDE4 subfamily³².

Although multiple PDEs are expressed in the CNS³³, PDE4 is ubiquitously present in astrocytes and neurons, where it is important to normal function and disease^{14, 16–18}. We reported that PDE4 was highly expressed in gliomas¹⁹ and that targeted inhibition with the selective PDE4 inhibitor rolipram³⁴ inhibited the intracranial growth of model GBM and medulloblastoma xenografts⁵. Interestingly, PDE4B was recently reported to be upregulated in medulloblastoma²³. Together with data cited above, these findings suggest that changes in PDE expression, especially of PDE4, may play a significant role in brain tumorigenesis.

Growth regulatory targets for cAMP signaling

Cyclic AMP can inhibit the growth of normal and neoplastic neural cell types through multiple mechanisms including regulation of cell cycle, apoptosis and differentiation. In astrocytes and astrocytoma cells, several studies have linked the anti-proliferative effects of cAMP to G1 cell cycle arrest. Cyclic AMP-dependent cell cycle arrest involves reduced expression of cyclins A and D1, and increased expression of the cyclin-dependent kinase inhibitors, p21Cip1 and p27Kip1^{6, 35, 36}.

Further, in GBM, gene expression profiling has identified genes belonging to matrix metalloproteases, signaling proteins, cell cycle modulators and developmental proteins as targets of cAMP³⁷. Notably, insulin-like growth factor binding protein-4 (IGFBP-4), a potent inhibitor of IGF-1 signaling and tumor proliferation, was identified as a key molecule upregulated by cAMP³⁸. In cerebellar granule precursor neurons, the cell of origin for a substantial subset of medulloblastoma³⁹, cAMP increased expression of the transcription factor *Lot-1*, which has recently been identified as a potent inhibitor of cerebellar granule precursor neuron proliferation and a promoter of differentiation^{40, 41}.

Cyclic AMP elevation has also been shown to mediate apoptosis via increased caspase activity in a wide variety of cells through multiple mediators such as p38 MAPK, PI3K, and the pro-apoptotic protein Bim^{42,43}.

Clinical applications

The growth inhibitory effects of cAMP prompted early speculation about the potential anti-cancer effects of cAMP and cAMP-elevating drugs. For example, cAMP analogs such as 8-chloro-cAMP or dibutyryl-cAMP have significant growth-inhibitory effects but are associated with dose-limiting toxicities⁴⁴. The focus of therapeutic cAMP elevation has been on modulating cAMP levels in other fashions. Thus, it is reasonable to consider modulation of GPCR functions and/or AC and PDE activity.

Targeting GPCRs is a focus of current clinical research. CXCR4 antagonism is currently in clinical trial for bone marrow stem cell mobilization and as a component of acute myeloid leukemia treatment⁴⁵. Brain tumor trials are planned but are not yet initiated. However, the abundance of GPCRs may render targeting of individual receptors less effective than targeting downstream mediators of their effects, like cAMP.

Multiple compounds are available for elevating cAMP through stimulation of its synthesis or inhibition of its degradation. General principles of pharmacology dictate that inhibition of degradation will have more potent and stable effects than stimulation of synthesis. Additional aspects of PDE pharmacology that enhance their targetability include: (i) common differences in cAMP synthetic and cAMP degradative capacities that result in there often being 10-fold greater capacity for cAMP hydrolysis than cAMP production, and (ii) the low concentration of intracellular cAMP (<1–10 M), which favors the efficacy of PDE competitive antagonists (see recent review in ref.⁴⁶). Thus, inhibition of cAMP degradation has been a longstanding focus in approaches to the therapeutic elevation of cAMP.

As described above, cAMP degradation is accomplished through the actions of multiple PDEs^{12, 47}. PDE function(s) in health and disease may involve regulating specific pools of cAMP, with spatial and temporal limits to their activity. Thus, successful therapeutic targeting of cAMP degradation might be accomplished either through broad PDE inhibition and global elevation of cAMP levels, or through highly specific PDE inhibitors, which could block the actions of single PDE isoforms and more precisely disrupt limited, (but crucial) aspects of cAMP signaling. Due to the highly conserved catalytic site within the PDE4 subfamily, this latter goal has proven difficult with catalytic site inhibitors. Recent insights into the structural basis for modulation of PDE enzymatic activity by its upstream conserved regions, phosphorylation and inhibitor binding, has yielded a novel class of allosteric inhibitors with PDE4D specificity²⁰. Isoform specific inhibition may also be possible with peptides and other compounds that target the unique amino-terminal domains of each isoenzyme^{34, 48}.

The history of cAMP-specific PDE targeting has been extensively reviewed⁴⁷. The initial clinical evaluation of PDE inhibition followed the recognition that caffeine and related methylxanthines (e.g. theophylline and aminophylline) have non-specific PDE inhibitory action. These agents have narrow therapeutic windows that are limited by their toxic effects on the CNS, cardiovascular and gastrointestinal systems. This, together with the rapidly expanding volume of information regarding the expression of specific PDE4 isoforms in individual cell types, and the involvement of PDE4 activity in inflammatory diseases such as asthma, COPD, arthritis and multiple sclerosis, fueled the development, preclinical and early clinical trial evaluations of multiple PDE4 inhibitors (Table 3).

Expression and functional studies provide a strong rationale for targeting PDE4 in brain tumors. While there are currently no planned clinical trials of PDE4 inhibition for brain tumors, it is our hope that one will be available soon. The weak PDE inhibitor pentoxifylline was in trial in combination with irradiation, cisplatin and mitomycin-C⁴⁹ as well as with irradiation and hydroxyurea as radiosensitizer in the treatment of GBM (Protocol ID NCI-95-C-0069). These combination therapies did not improve disease control and were associated with excessive CNS and gastrointestinal toxicities. Thus more specific and potent PDE inhibitors must be further evaluated.

Concluding remarks

The time is ripe for the application of cAMP-elevating drugs to cancer therapeutics. PDEs have been implicated in carcinogenesis and tumor progression for a variety of tumor types including: prostate cancer⁵⁰, brain tumors^{5, 11, 19}, hematological malignancies^{51, 52}, colon cancer⁵³, melanoma⁵⁴, as well as in the enhancement of drug delivery⁵⁵. Finally, various PDE inhibitors have been or are being actively clinically evaluated (Table 3).

However, several important issues must be addressed prior to the successful incorporation of PDE inhibition into cancer therapeutic regimens. First, the development of PDE inhibitors, especially PDE4 inhibitors, has been powerfully guided by the desire to reduce the gastrointestinal side effects of the catalytic site directed competitive antagonists. Overcoming these toxicities may require refinement in this class of inhibitors or advancement of alternative inhibitors such as allosteric or isoform-specific inhibitors^{20, 34, 48}.

Regardless, in cancer therapeutics, nausea and vomiting are common, anticipated, and well controlled through the use of myriad established and potent anti-emetics. The risk-benefit analysis for the use of effective, yet emetogenic agents, is overwhelmingly in favor of their use. Emetogenicity should not limit the advancement of effective agents as anti-cancer medications. In this regard, broad screening of any PDE inhibitor for which expression data supports its use is warranted, with a focus on anti-tumor activity alone.

Although isoform-specific inhibition could be appropriate for some cancers, there are data to implicate multiple cAMP-specific PDEs in the promotion of neoplastic growth. We showed that PDE4A1 can stimulate gliomagenesis; it is possible that other PDE4 isoenzymes, or even other cAMP PDEs could do the same^{11, 19}. PDE4D has been demonstrated to be important for prostate carcinogenesis⁵⁰ and PDE7 appears to be required for leukemic cell growth *in vitro*⁵². Thus, while there is a rationale for evaluating isoform-specific inhibitors as cancer therapeutics, broad PDE inhibition could be required for maximal anti-tumor effects. The feasibility of pan-PDE4 inhibition was demonstrated in clinical trials of rolipram for depression⁵⁶.

In addition, it is important to consider the multiple potential cellular and intracellular targets for PDE inhibition in cancer therapeutics. Although data indicate that tumor cells are sensitive to cAMP elevation, cAMP also regulates endothelial cell activity⁵⁷ and CNS inflammatory responses⁵⁸, both potentially powerful modulators of brain tumor growth. It may also be necessary to consider how precise genetic profiles of cancer cells impact on PDE inhibitor effects. For example, astrocytomas commonly possess genetic alterations that activate the MAPK pathway, a driver of their growth^{59, 60}. These oncogenetic events include: amplification or mutational activation of cell surface receptors like the EGF and PDGF receptors, mutational activation of K-RAS, B-RAF, or loss of neurofibromin. Cyclic AMP regulates the MAPK pathway at multiple levels between the cell surface and the nucleus^{34, 61} (Figure 3). Thus, the tumor-specific profile of genetic alterations could impact on the efficacy of cAMP elevation. In addition, the regulation of PDE4 activity by the ERK

and PKA pathways must also be considered^{20, 34}. Inhibition and stimulation of PDE4 activity by ERK and PKA are different for long and short forms. Therefore the integrated response of the full cellular complement of PDE4 to any inhibitor may be difficult to predict. Any clinical trial of PDE4 inhibitors should include efforts to profile not only the known oncogenetic events but also the PDE complement.

Finally, the lessons learned from comparing the activity of aminophylline and theophylline to specific PDE4 inhibitors in the treatment of asthma suggests that the combination of PDE inhibition with other target modulation might be most effective^{47, 62}. Theophylline and aminophylline have been in clinical use for nearly a century. Their mechanism of action is incompletely understood but they are known to be weak pan-PDE inhibitors as well as adenosine receptor antagonists⁶³. The potential importance of this combined activity was recently rediscovered in a high throughput drug screen for anti-leukemic cell activity. In this study the combination of PDE7 inhibition and adenosine receptor A2 antagonism was identified as possessing superior anti-tumor effect⁶⁴. It is possible that newer generation agents with these combined properties could maintain or enhance their activity and reduce their toxicity.

In sum, we have made enormous strides in understanding the complexity of cAMP regulation of growth and the role that PDE4 plays in specifying cAMP activity. Multiple PDE4 inhibitors, with defined activity, have been evaluated in preclinical and clinical studies. The rationale and the tools for cancer application are available, and the desire is strong to usher in this new era with reasonable hopes for breakthroughs.

Acknowledgments

This work was supported by NCI/NIH RO1CA118389 (JBR).

References

1. Sutherland EW. Nobel prize in physiology or medicine 1971: the action of hormones outlined. *Lakartidningen*. 1971; 68:4991–4995. [PubMed: 4332679]
2. Savai R, et al. Targeting cancer with phosphodiesterase inhibitors. *Expert Opin Investig Drugs*. 2010; 19:117–131.
3. Furman MA, Shulman K. Cyclic AMP and adenylyl cyclase in brain tumors. *J Neurosurg*. 1977; 46:477–483. [PubMed: 191576]
4. Warrington NM, et al. Spatiotemporal differences in CXCL12 expression and cyclic AMP underlie the unique pattern of optic glioma growth in neurofibromatosis type 1. *Cancer Res*. 2007; 67:8588–8595. [PubMed: 17875698]
5. Yang L, et al. Blocking CXCR4-Mediated Cyclic AMP Suppression Inhibits Brain Tumor Growth In vivo. *Cancer Res*. 2007; 67:651–658. [PubMed: 17234775]
6. Chen TC, et al. The type IV phosphodiesterase inhibitor rolipram induces expression of the cell cycle inhibitors p21(Cip1) and p27(Kip1), resulting in growth inhibition, increased differentiation, and subsequent apoptosis of malignant A-172 glioma cells. *Cancer Biol Ther*. 2002; 1:268–276. [PubMed: 12432276]
7. Schmidt AL, et al. BDNF and PDE4, but not the GRPR, regulate viability of human medulloblastoma cells. *J Mol Neurosci*. 2009; 40:303–310. [PubMed: 19642024]
8. Lelievre V, et al. Disruption of the PACAP gene promotes medulloblastoma in ptc1 mutant mice. *Dev Biol*. 2008; 313:359–370. [PubMed: 18036580]
9. Liu X, et al. Induction of cell cycle arrest at G1 and S phases and cAMP-dependent differentiation in C6 glioma by low concentration of cycloheximide. *BMC Cancer*. 10:684. [PubMed: 21159181]
10. Rubin JB, Gutmann DH. Neurofibromatosis type 1 - a model for nervous system tumour formation? *Nat Rev Cancer*. 2005; 5:557–564. [PubMed: 16069817]

11. Warrington NM, et al. Cyclic AMP suppression is sufficient to induce gliomagenesis in a mouse model of neurofibromatosis-1. *Cancer Res.* 2010; 70:5717–5727. [PubMed: 20551058]
12. Huston E, et al. cAMP phosphodiesterase-4A1 (PDE4A1) has provided the paradigm for the intracellular targeting of phosphodiesterases, a process that underpins compartmentalized cAMP signalling. *Biochem Soc Trans.* 2006; 34:504–509. [PubMed: 16856845]
13. Keravis T, Lugnier C. Cyclic nucleotide phosphodiesterases (PDE) and peptide motifs. *Curr Pharm Des.* 2010; 16:1114–1125. [PubMed: 20030615]
14. Kelly MP, Brandon NJ. Differential function of phosphodiesterase families in the brain: gaining insights through the use of genetically modified animals. *Prog Brain Res.* 2009; 179:67–73. [PubMed: 20302819]
15. Itoh T, et al. PET measurement of the in vivo affinity of 11C-(R)-rolipram and the density of its target, phosphodiesterase-4, in the brains of conscious and anesthetized rats. *J Nucl Med.* 2009; 50:749–756. [PubMed: 19372471]
16. Zhang HT. Cyclic AMP-specific phosphodiesterase-4 as a target for the development of antidepressant drugs. *Curr Pharm Des.* 2009; 15:1688–1698. [PubMed: 19442182]
17. Siuciak JA. The role of phosphodiesterases in schizophrenia : therapeutic implications. *CNS Drugs.* 2008; 22:983–993. [PubMed: 18998737]
18. Braun NN, et al. Expression of phosphodiesterase 4 is altered in the brains of subjects with autism. *Neuroreport.* 2007; 18:1841–1844. [PubMed: 18090323]
19. Goldhoff P, et al. Targeted inhibition of cyclic AMP phosphodiesterase-4 promotes brain tumor regression. *Clin Cancer Res.* 2008; 14:7717–7725. [PubMed: 19047098]
20. Burgin AB, et al. Design of phosphodiesterase 4D (PDE4D) allosteric modulators for enhancing cognition with improved safety. *Nat Biotechnol.* 2010; 28:63–70. [PubMed: 20037581]
21. Jacobitz S, et al. Role of conserved histidines in catalytic activity and inhibitor binding of human recombinant phosphodiesterase 4A. *Mol Pharmacol.* 1997; 51:999–1006. [PubMed: 9187266]
22. Racagni G, et al. Cyclic nucleotides in experimental and human brain tumors. *J Neurooncol.* 1983; 1:61–67. [PubMed: 6086852]
23. Northcott PA, et al. Medulloblastoma Comprises Four Distinct Molecular Variants. *J Clin Oncol.* 2010
24. Domanska UM, et al. The chemokine network, a newly discovered target in high grade gliomas. *Crit Rev Oncol Hematol.* 2010
25. Sciume G, et al. Chemokines and glioma: invasion and more. *J Neuroimmunol.* 2010; 224:8–12. [PubMed: 20656128]
26. Bian XW, et al. Preferential expression of chemokine receptor CXCR4 by highly malignant human gliomas and its association with poor patient survival. *Neurosurgery.* 2007; 61:570–578. discussion 578–579. [PubMed: 17881971]
27. Calatuzzolo C, et al. Prognostic value of CXCL12 expression in 40 low-grade oligodendrogliomas and oligoastrocytomas. *Cancer Biol Ther.* 2006; 5:827–832. [PubMed: 16760646]
28. Rubin JB, et al. A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. *Proc Natl Acad Sci U S A.* 2003; 100:13513–13518. [PubMed: 14595012]
29. Zagzag D, et al. Hypoxia- and vascular endothelial growth factor-induced stromal cell-derived factor-1 α /CXCR4 expression in glioblastomas: one plausible explanation of Scherer's structures. *Am J Pathol.* 2008; 173:545–560. [PubMed: 18599607]
30. Kenig S, et al. Glioblastoma and endothelial cells cross-talk, mediated by SDF-1, enhances tumour invasion and endothelial proliferation by increasing expression of cathepsins B, S, and MMP-9. *Cancer Lett.* 2010; 289:53–61. [PubMed: 19700239]
31. Conti M, Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu Rev Biochem.* 2007; 76:481–511. [PubMed: 17376027]
32. Houslay MD. Underpinning compartmentalised cAMP signalling through targeted cAMP breakdown. *Trends Biochem Sci.* 2010; 35:91–100. [PubMed: 19864144]
33. Kleppisch T. Phosphodiesterases in the central nervous system. *Handb Exp Pharmacol.* 2009:71–92. [PubMed: 19089326]

34. Houslay MD, et al. Keynote review: phosphodiesterase-4 as a therapeutic target. *Drug Discov Today*. 2005; 10:1503–1519. [PubMed: 16257373]
35. Gagelin C, et al. Inhibition of G1 cyclin expression and G1 cyclin-dependent protein kinases by cAMP in an astrocytic cell line. *Biochem Biophys Res Commun*. 1994; 205:923–929. [PubMed: 7999133]
36. Balmanno K, et al. DeltaRaf-1:ER* bypasses the cyclic AMP block of extracellular signal-regulated kinase 1 and 2 activation but not CDK2 activation or cell cycle reentry. *Mol Cell Biol*. 2003; 23:9303–9317. [PubMed: 14645540]
37. Hill JJ, et al. Identification of secreted proteins regulated by cAMP in glioblastoma cells using glycopeptide capture and label-free quantification. *Proteomics*. 2009; 9:535–549. [PubMed: 19137551]
38. Moreno MJ, et al. Insulin-like growth factor binding protein-4 (IGFBP-4) is a novel anti-angiogenic and anti-tumorigenic mediator secreted by dibutyryl cyclic AMP (dB-cAMP)-differentiated glioblastoma cells. *Glia*. 2006; 53:845–857. [PubMed: 16586492]
39. Yang ZJ, et al. Medulloblastoma can be initiated by deletion of Patched in lineage-restricted progenitors or stem cells. *Cancer Cell*. 2008; 14:135–145. [PubMed: 18691548]
40. Fila T, et al. Lot1 is a key element of the pituitary adenylate cyclase-activating polypeptide (PACAP)/cyclic AMP pathway that negatively regulates neuronal precursor proliferation. *J Biol Chem*. 2009; 284:15325–15338. [PubMed: 19346254]
41. Contestabile A, et al. Cyclic AMP-mediated regulation of transcription factor Lot1 expression in cerebellar granule cells. *J Biol Chem*. 2005; 280:33541–33551. [PubMed: 16061485]
42. Ahn YH, et al. 8-Chloro-cyclic AMP-induced growth inhibition and apoptosis is mediated by p38 mitogen-activated protein kinase activation in HL60 cells. *Cancer Res*. 2005; 65:4896–4901. [PubMed: 15930311]
43. Zhang L, Insel PA. The pro-apoptotic protein Bim is a convergence point for cAMP/protein kinase A- and glucocorticoid-promoted apoptosis of lymphoid cells. *J Biol Chem*. 2004; 279:20858–20865. [PubMed: 14996839]
44. Vitale G, et al. A new therapeutic strategy against cancer: cAMP elevating drugs and leptin. *Cancer Biol Ther*. 2009; 8:1191–1193. [PubMed: 19550148]
45. DiPersio JF, et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. *J Clin Oncol*. 2009; 27:4767–4773. [PubMed: 19720922]
46. Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev*. 2006; 58:488–520. [PubMed: 16968949]
47. Press NJ, Banner KH. PDE4 inhibitors - a review of the current field. *Prog Med Chem*. 2009; 47:37–74. [PubMed: 19328289]
48. Bruno O, et al. New selective phosphodiesterase 4D inhibitors differently acting on long, short, and supershort isoforms. *J Med Chem*. 2009; 52:6546–6557. [PubMed: 19827751]
49. Stewart DJ, et al. Cranial radiation and concomitant cisplatin and mitomycin-C plus resistance modulators for malignant gliomas. *J Neurooncol*. 1997; 32:161–168. [PubMed: 9120546]
50. Rahrman EP, et al. Identification of PDE4D as a proliferation promoting factor in prostate cancer using a Sleeping Beauty transposon-based somatic mutagenesis screen. *Cancer Res*. 2009; 69:4388–4397. [PubMed: 19401450]
51. Meyers JA, et al. Chronic lymphocytic leukemia and B and T cells differ in their response to cyclic nucleotide phosphodiesterase inhibitors. *J Immunol*. 2009; 182:5400–5411. [PubMed: 19380787]
52. Zhang L, et al. Cyclic nucleotide phosphodiesterase profiling reveals increased expression of phosphodiesterase 7B in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A*. 2008; 105:19532–19537. [PubMed: 19033455]
53. McEwan DG, et al. Chemoresistant KM12C colon cancer cells are addicted to low cyclic AMP levels in a phosphodiesterase 4-regulated compartment via effects on phosphoinositide 3-kinase. *Cancer Res*. 2007; 67:5248–5257. [PubMed: 17545604]
54. Murata T, et al. Expression and role of phosphodiesterase 5 in human malignant melanoma cell line. *Anticancer Res*. 30:355–358. [PubMed: 20332439]

55. Black KL, et al. PDE5 inhibitors enhance tumor permeability and efficacy of chemotherapy in a rat brain tumor model. *Brain Res.* 2008; 1230:290–302. [PubMed: 18674521]
56. Bielekova B, et al. Treatment with the phosphodiesterase type-4 inhibitor rolipram fails to inhibit blood–brain barrier disruption in multiple sclerosis. *Mult Scler.* 2009; 15:1206–1214. [PubMed: 19776093]
57. Wang J, et al. Intrinsic sex-specific differences in microvascular endothelial cell phosphodiesterases. *Am J Physiol Heart Circ Physiol.* 2010; 298:H1146–1154. [PubMed: 20139324]
58. Dejda A, et al. Neuroprotective potential of three neuropeptides PACAP, VIP and PHI. *Pharmacol Rep.* 2005; 57:307–320. [PubMed: 15985713]
59. Thaker NG, Pollack IF. Molecularly targeted therapies for malignant glioma: rationale for combinatorial strategies. *Expert Rev Neurother.* 2009; 9:1815–1836. [PubMed: 19951140]
60. Kesari S, et al. Targeted molecular therapy of malignant gliomas. *Curr Oncol Rep.* 2006; 8:58–70. [PubMed: 16464405]
61. Dumaz N, Marais R. Integrating signals between cAMP and the RAS/RAF/MEK/ERK signalling pathways. Based on the anniversary prize of the Gesellschaft für Biochemie und Molekularbiologie Lecture delivered on 5 July 2003 at the Special FEBS Meeting in Brussels. *FEBS J.* 2005; 272:3491–3504. [PubMed: 16008550]
62. Somerville LL. Theophylline revisited. *Allergy Asthma Proc.* 2001; 22:347–351. [PubMed: 11775391]
63. Hansel TT, et al. Theophylline: mechanism of action and use in asthma and chronic obstructive pulmonary disease. *Drugs Today (Barc).* 2004; 40:55–69. [PubMed: 14988770]
64. Rickles RJ, et al. Adenosine A2A receptor agonists and PDE inhibitors: a synergistic multitarget mechanism discovered through systematic combination screening in B-cell malignancies. *Blood.* 116:593–602. [PubMed: 20382846]
65. Kamenetsky M, et al. Molecular details of cAMP generation in mammalian cells: a tale of two systems. *J Mol Biol.* 2006; 362:623–639. [PubMed: 16934836]
66. Sadana R, Dessauer CW. Physiological roles for G protein-regulated adenylyl cyclase isoforms: insights from knockout and overexpression studies. *Neurosignals.* 2009; 17:5–22. [PubMed: 18948702]
67. Visel A, et al. Comprehensive analysis of the expression patterns of the adenylate cyclase gene family in the developing and adult mouse brain. *J Comp Neurol.* 2006; 496:684–697. [PubMed: 16615126]
68. Omori K, Kotera J. Overview of PDEs and their regulation. *Circ Res.* 2007; 100:309–327. [PubMed: 17307970]
69. Baillie GS, et al. TAPAS-1, a novel microdomain within the unique N-terminal region of the PDE4A1 cAMP-specific phosphodiesterase that allows rapid, Ca²⁺-triggered membrane association with selectivity for interaction with phosphatidic acid. *J Biol Chem.* 2002; 277:28298–28309. [PubMed: 11994273]

Figure 1. PDE4 subfamily: isoforms and intracellular localization of PDE4A1

The PDE4 subfamily of PDE includes four genes (A, B, C and D) each of which can generate multiple isoenzymes. Isoenzymes may be of three distinct types: (i) long, (ii) short, or (iii) supershort, based on the configuration of regulatory domains located between the N-terminus (N-T) (isoform specific) and the catalytic unit. Long forms contain upstream conserved regions (UCR) 1 and 2. Short forms contain only UCR2, and the super-short forms contain a truncated UCR2. PDE4A1 is a supershort isoform that is localized to the trans-golgi apparatus. The unique N-T of PDE4A1 consists of 2 helices separated by a hinge region. Binding of Ca^{+2} to Asp 21 in the TAPAS-1 domain of helix 2 results in the membrane insertion of the N-T while Asp 5 in LxDFF domain of helix 1 responds to intracellular Ca^{+2} changes by either targeting the enzyme to the trans-golgi membrane or its relocation to cytosolic organelles in a Phospholipase D (PLD) dependant manner. Overall, the spatiotemporal distribution of PDE4A1 in the cell is tightly regulated by intracellular Ca^{++} as well as Phosphatidic acid (PA) generation by PLD^{12, 69}.

Figure 2. Golgi localized PDE4 is tumorigenic

(A) Phosphodiesterase 4A1 (PDE4A1) is localized to the trans-Golgi, potentially creating a gradient of cAMP from low (yellow) to high (blue). (B) Ectopic expression of PDE4A1 induced glioma formation in a mouse model of Neurofibromatosis 1 (NF1). Lentivirus encoding catalytically active (PDE4A1), but not catalytically dead (PDE4A1-H229Q) induced cortical gliomas in a mouse model of NF1-associated optic pathway glioma. Ectopic tumors were evident as increased expression of the tumor-associated transcription factor Olig2 (brown) compared to expression in adjacent uninvolved areas of the brain. These findings suggest that PDE4A1-mediated cAMP suppression is sufficient to induce glioma in the setting of NF1. (C) Overexpression of PDE4A1 in intracranial xenografts of either Daoy medulloblastoma cells or U87 glioblastoma cells accelerated their growth. Shown are bioluminescence images of control and PDE4A1 expressing tumors. Increased area of luminosity is correlated with increased growth. These data indicate that PDE4A1-mediated cAMP suppression is sufficient to accelerate brain tumor growth.

Figure 3. Growth targets of cAMP Signaling

Intracellular activation of AC converts ATP to cAMP resulting in modulation of activity in growth promoting pathways. Some of the multiple downstream cascades that regulate growth and are modulated by cAMP are shown. The majority of cAMP effects on signal transduction have been ascribed to cAMP-induced activation of PKA. However, PKA-independent actions of cAMP have also been demonstrated through specific activation of exchange proteins directly activated by cAMP (EPAC 1 and 2). Effects of cAMP are cell type-specific and often hinge upon differential expression of RAF isoforms. These critical regulators of the mitogen activated protein kinase (MAPK) pathway can either be stimulated by cAMP (RAF-B) or inhibited by cAMP (RAF-C). Prolonged stimulation of cAMP might cause transcriptional activation of targets such as AP-1 and Lot-1, which can regulate cell survival, and repression of cell cycle regulators (e.g. cyclins and cyclin dependant kinases) leading to growth arrest. Finally, elevation of intracellular cAMP levels has also been shown to induce apoptotic cell death through PKA, protein kinase C (PKC), EPAC and Akt-mediated pathways.

Table 1

Transmembrane Adenylyl Cyclase: Expression and Regulation

Isoform	Function	Distribution	Regulation by G protein			Regulation by Ca ²⁺		Regulation by protein kinase		Class
			Gαi	Gαs	Gαγ			Stimulatory	Inhibitory	
AC1	Learning, memory, synaptic plasticity, opiate withdrawal	Brain	Inhibition	Stimulation	Inhibition	Stimulation (CaM)		PKCa RTK	CaMK IV	A
AC2		Brain, lung, heart, skeletal muscle	No effect	Stimulation	Stimulation	No effect		PKC		B
AC3	Olfaction, sperm function	Brain, lung, heart, testis, pancreas, Olfactory epithelium	Inhibition	Stimulation	No effect	Stimulation (CaM)		PKCa	CaMK II	A
AC4		Widespread	No effect	Stimulation	Stimulation	No effect			PKCa	B
AC5	Heart contraction, motor co-ordination	Lung, liver, heart, striatum, testis, widespread	Inhibition	Stimulation	No effect	Inhibition		PKCa PKCγ	PKA	C
AC6	Heart contraction, calcium sensitivity	Heart, kidney, Widespread	Inhibition	Stimulation	No effect	Inhibition		PKCa PKCγ	PKA PKCδ PKCε	C
AC7	Ethanol dependence	Widespread	No effect	Stimulation	No effect	No effect		PKCa		B
AC8	Learning, memory, synaptic plasticity, Opiate withdrawal	Brain, pancreas	Inhibition	Stimulation	Inhibition	Stimulation (CaM)				A
AC9		Testis, widespread	Inhibition	Stimulation	No effect	Inhibition (Calcieneurin)			PKC	D

Abbreviations: PKC: Protein kinase C, CaM: Calmodulin, RTK: Receptor Tyrosine Kinase, CaMK: Ca²⁺/Calmodulin dependent protein kinase, References: 65-67

Table 2**PDE4: Expression and Regulation**

PDE4 Sub-family	PDE4A	PDE4B	PDE4C	PDE4D
Tissue of Expression	Widespread, rich in brain	Widespread	Widespread but low in lung and absent in blood	Widespread but PDE4D4 and 4D6 are brain specific
Substrate	All cAMP specific			
Long form	4A4B, 4A8, 4A10, 4A11	4B1, 4B3, 4B4	4C1, 4C2 & 4C3	4D3, 4D4, 4D5, 4D7, 4D8, 4D9 & 4D11
Short form		4B2, 4B5		4D1
Super short form	4A1			4D2, 4D6 and 4D10
Regulation	All can be phosphorylated by ERK but with different outcomes: short isoforms are activated, while long and super short isoforms are negatively regulated by ERK phosphorylation. Long isoforms can also be activated by PKA phosphorylation.			
Regulatory protein	ERK B-arrestin AIP (PDE4A4/5) P62 (PDE4A4/5) P75NTR (PDE4A4/5) Proteins with SH3 domain DISC1	ERK B-arrestin DISC1	ERK B-arrestin DISC1	ERK B-arrestin mAKAP (PDE4D3) RACK1 (PDE4D5) Myomegalin (PDE4D3) AKAP450 (PDE4D3) Proteins with SH3 domain EPAC1 (PDE4D3) β 1-Adrenoceptor DISC1

Abbreviations: ERK: Extracellular signal-regulated kinases, mAKAP: Muscle specific A-kinase anchoring protein, RACK1: Receptor for activated C kinase 1, AKAP: A Kinase Anchoring Protein, EPAC: Exchange protein directly activated by cAMP, p75NTR: p75 neurotrophin receptor, DISC1: Disrupted-in-schizophrenia 1.

References: 32, 68

Table 3

Clinical Trials of PDE4 inhibitors

Drug	Indication	Phase	Results
Rolipram	Depression	3	>200 patients in 5 clinical trials. A single study showed some efficacy. Associated with less anti-cholinergic side effects but increased GI complaints.
Roflumilast	COPD/Asthma	3	4545 patients treated in multiple trials. Small improvements in both pre- and post-bronchodilator FEV ₁ . Reductions in relapse rates. There were no improvements in SGRQ (St Georges Respiratory Questionnaire). Diarrhea, nausea and weight loss were the most common reported side effects.
Cilomilast	COPD/Asthma	3	>6000 patients treated in multiple clinical trials. Small improvement in measured FEV ₁ . No significant effect on clinical or inflammatory scores. In all studies there were more GI complaints in the treatment group.
Tetomilast	Crohn's Disease/IBD	2	186 patients. No improvements in remission but there was a possibility of improved disease activity scores in those with the most severe disease. GI complaints were more common in the treatment group.
Oglemilast	COPD	2	No reports
MEM1414	Alzheimer's Disease	1	No reports
IPL-455903	Memory Enhancement	1	No reports
GRC-4039	Rheumatoid Arthritis	1	No reports

Reviewed in Press⁴⁷.