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Review

Short PDE4 Isoforms as Drug Targets in Disease

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Abstract

The second messenger, cyclic adenosine monophosphate (cAMP), is a master regulator of signal transduction that maintains cell homeostasis. A fine balance between cAMP synthesis by adenylyl cyclase and degradation by phosphodiesterases (PDEs) underpins receptor-specific responses. As multiple receptors rely on cAMP for signaling, PDEs shape three-dimensional, localized gradients of the cyclic nucleotide to drive appropriate signaling cascades. Of the 11 PDE families, PDE4, which comprises long, short, and supershort isoforms and a dead-short isoform, is of great interest due to its implication in disease. Aberrant PDE4 expression and post-translational modifications are hallmarks of several clinical indications for which curative treatment is not yet available. While some PDE4-specific small molecule inhibitors directed against the active site are approved for clinical use, they are limited by severe side effects owing to the high degree of conservation of the catalytic domain between over 20 unique isoforms. Some attempts to use the different modular structure that exists between long and shorter isoforms are now bearing success. However, these inhibitors are exclusively aimed at PDE4 long isoforms, which have been the focus of the majority of research in this area. Here, we have summarised literature on the lesser-studied short PDE4 isoforms and provide a record of the discovery, regulation, and disease relevance of this class of enzymes that represent an untapped target for specific inhibition in the future.

Keywords: phosphodiesterase; cyclic AMP; short isoform; inflammation; multiple sclerosis; Alzheimer's disease; traumatic brain injury; cancer; chronic obstructive pulmonary disease; drug target

1. Introduction

The first identified second messenger, 3′, 5′-cyclic adenosine monophosphate (cAMP), was isolated by Earl Sutherland in 1971 and has proved to be a vital transducer of intracellular signaling in response to internal and external cues [1]. Following its synthesis by adenylyl cyclase, cAMP diffuses rapidly throughout the cell to activate a myriad of signaling pathways by binding downstream effectors, including protein kinase A (PKA), the guanine exchange factor exchange protein directly activated by cAMP (EPAC), cyclic nucleotide-gated ion channels, and Popeye-domain containing proteins [2–5]. Phosphodiesterases (PDEs) hydrolyze cyclic nucleotides to ensure compartment- and signal-specific activation of downstream effectors. Compartmentalized cAMP signaling is underpinned by the formation of signalosomes: multiprotein complexes of cAMP effectors, anchoring proteins (e.g., A-kinase-anchoring-protein), and specific subsets of PDEs [6]. In the basal state, PDEs within signalosomes prevent activation of cAMP effectors; when cAMP levels increase in response to an agonist, localized PDE activity is swamped, and cAMP can initiate downstream signaling by binding the effector [7].

Phosphodiesterase activity is indispensable for maintaining discrete cellular responses to the multitude of signals a cell receives, and dysregulated PDE expression is associated with numerous pathologies of the cardiovascular, nervous, and immune systems. Importantly, altered PDE activity is also a contributing factor to the development and progression of malignant disease. Of the 11 families of PDEs, the PDE4 family is the largest: it comprises over 20 isoforms, encoded by genes A, B, C, and D, which are classified as long, short, supershort, or dead-short, depending on the presence and length of the upstream conserved region (UCR) 1 and 2 domains, unique to the PDE4 hydrolases (Fig. 1) [8].

In long isoforms, the carboxy-terminus of UCR1 interacts with the amino-terminus of UCR2 to form a regulatory unit which, in the resting PDE4 state, occludes the catalytic domain of a second PDE4 (partner in a dimer) to downregulate its activity. Thus, UCRs not only regulate cAMP hydrolysis but they contribute to the quaternary, dimeric structure of all long PDE4 isoforms. Phosphorylation of a classical PKA consensus motif (RRxS) in UCR1 by PKA enhances PDE4 activity through the disruption of the UCR1-UCR2 inhibitory conformation [9–12] (Fig. 2). Conversely, phosphorylation of the extracellular signal-regulated kinase (ERK2) consensus motif (PxS/TP) within the catalytic domain results in long PDE4B, PDE4C, and PDE4D inhibition [11,13] (Fig. 2). In contrast to long isoforms, which have both UCRs, short isoforms only have UCR2, supershort isoforms lack UCR1 and have a truncated UCR2, and dead-short isoforms have no UCRs and a truncated catalytic domain [14,15]. In complementary fashion to long-form activation, the activation of CREB by PKA can result in the

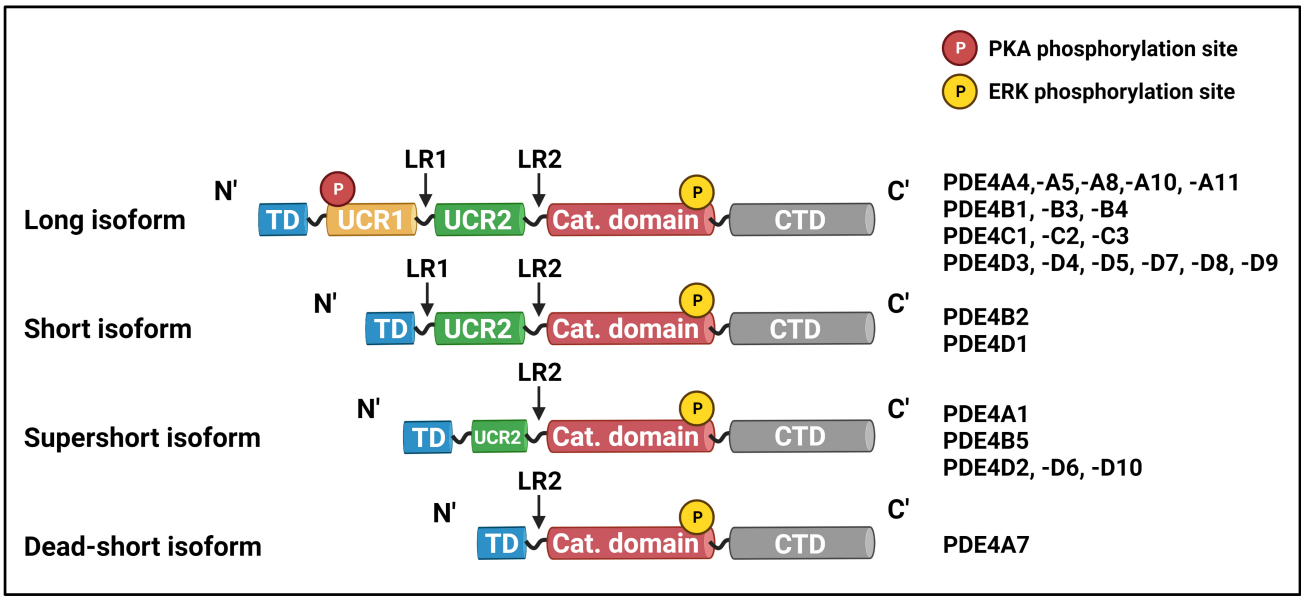

Fig. 1. PDE4 isoform structure and classification. Depending on the length of their N-terminal region, members of the PDE4 family are long, short, supershort, or dead-short. All PDE4 isoforms are listed on the right of their respective class and structure. PDE4, phosphodiesterase 4; TD, targeting domain; UCR, upstream conserved region; LR, linker region; Cat. Domain, catalytic domain; CTD, C′ terminal domain; PKA, protein kinase A; ERK, extracellular signal-regulated kinase. Created with BioRender.com.

enhanced transcription/translation of short and supershort isoforms (SASSI), ensuring only transient increases in cellular cAMP concentrations (Fig. 2). The function, expression, and role of long PDE isoforms in disease have been extensively reviewed elsewhere [6,16], though no such undertaking has been completed for human PDE4 SASSI. This review aims to bring together current research on the roles of PDE4 SASSI in cellular homeostasis and disease.

2. Discovery

The *Drosophila melanogaster* (*D. melanogaster*) *dunce* (*dnc*) gene, which encodes a PDE hydrolase, was the first one identified as necessary for normal fly behavioral development: *dnc* mutants are characterized by short-term memory and learning deficiencies [17]. Screening of rat libraries with a probe representing *dnc* enabled the identification and cloning of the rat *dnc* homolog, *ratdnc-1*. Of the four cDNA clones isolated in the study, PDE4A1 (formerly RD1) was the first identified supershort isoform [18]. In an investigation of the structure and function of the rat *dnc* homolog, a rat testis cDNA library was screened using a cDNA clone of the *D. melanogaster dunce* PDE. This study yielded four groups of clones (*ratPDE1-4*) encoded by four different genes [19]. Shortly after, in a study of hormonal regulation of PDEs, ratPDE3.1 and ratPDE3.2 (homologs of human *PDE4D1* and *PDE4D2* below) were characterized [20]. In search of human *dnc* homologs, Bolger and colleagues isolated the cDNA of the four human genes from the PDE4 family: *PDE4A*, *PDE4B*, *PDE4C*, and *PDE4D* (formerly DPDE1 through 4) [8]. Subsequently, the first human short isoform to be uncovered was PDE4B2 (formerly hb-PDE1a): its cDNA was isolated by screening a human frontal cortex cDNA library using a monocyte cDNA fragment encoding a PDE4. DNA sequencing indicated that *PDE4B2* has a truncated open reading frame and is homologous only to the 3′-end of the monocyte PDE4 [21]. Soon after, studies on rat Sertoli, thyroid, and brain cells provided evidence for the existence of PDE4D SASSI, PDE4D1, and PDE4D2, resulting from alternative splicing [22,23]. Their human homologs were identified in human peripheral mononuclear cells [9]. The cDNA encoding the dead-short isoform, PDE4A7 (formerly HSPDE4A8), was isolated from Jurkat T cells; unlike the long isoform, PDE4A4, it contained an insert in the catalytic domain, making it catalytically inactive [24]. Based on earlier work from Davis *et al*., 1989 [18], PDE4A1 was shown to be expressed in the human cerebellum [25]. In a study exploring further isoforms encoded by the *PDE4D* gene, murine *PDE4D6* was cloned and characterized: it was shown to lack half of the UCR2 region, making it a supershort PDE4 [26]. Intriguingly, the alignment of mouse-expressed sequence-tagged transcripts to the human genome uncovered the supershort PDE4B5 isoform, which was shown to have 16 N-terminal residues identical to those of PDE4D6 [27]. Further experimental work on murine *PDE4D* led to the cloning of the supershort PDE4D10 isoform [28]. In summary, the human PDE4 SASSI discovered to date include *PDE4A1*, *PDE4A7*, *PDE4B2*, *PDE4B5*, *PDE4D1*, *PDE4D2*, *PDE4D6*, and *PDE4D10*.

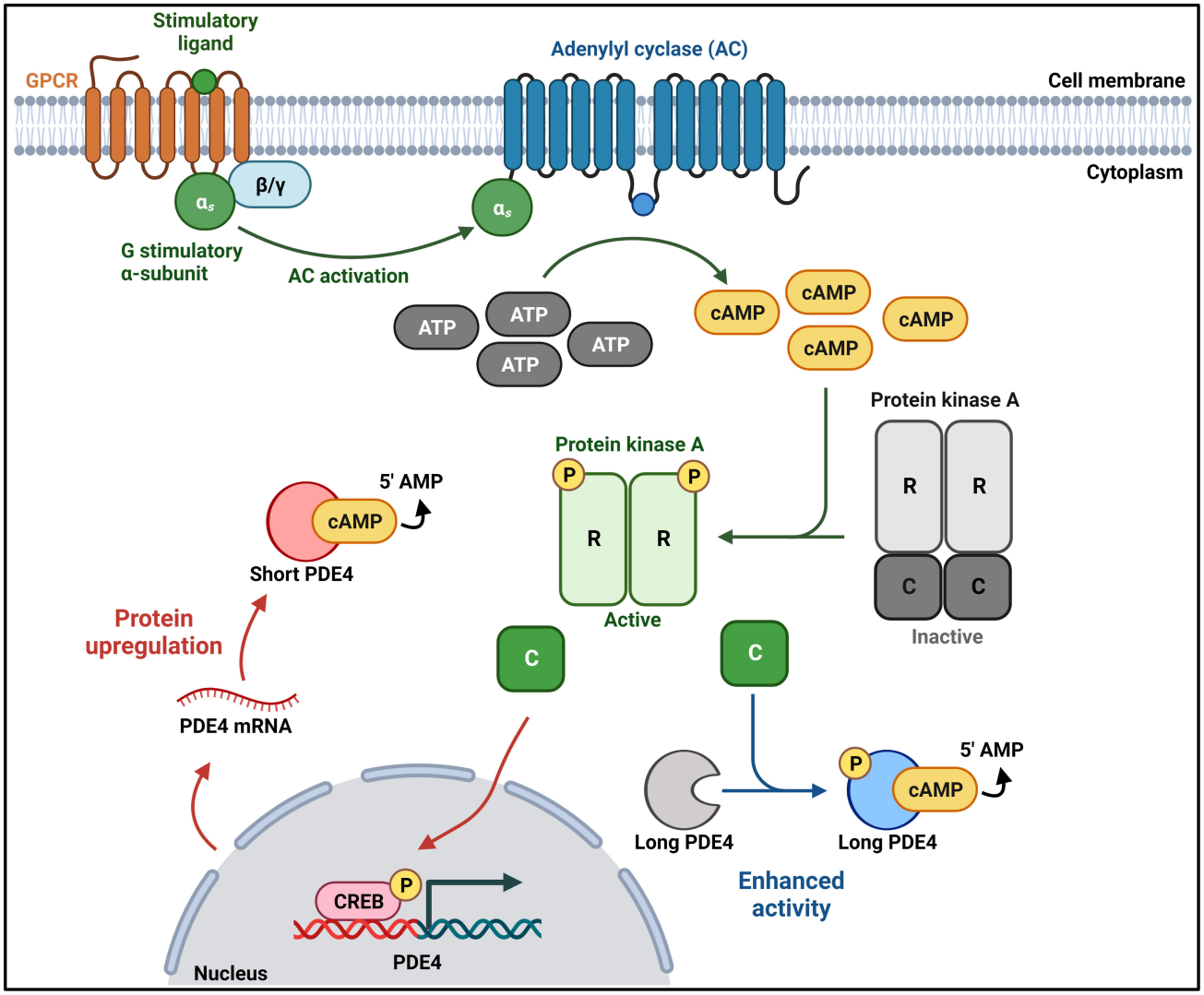

Fig. 2. Long and short PDE4 regulation. G-protein-coupled receptor (GPCR) activation induces adenylyl cyclase (AC) activation and the production of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). Increased cAMP levels lead to phosphorylation and activation of Protein kinase A (PKA). Conformational changes induced by the phosphorylation of PKA lead to the dissociation of the PKA catalytic (C) subunit from the regulatory (R) subunit dimer. The catalytic subunit can phosphorylate long PDE4 isoforms to enhance their activity and phosphorylate the cAMP-response element binding (CREB) protein to enhance short, supershort, and dead-short *PDE4* isoform transcription, which in turn drives upregulated protein expression. Created with BioRender.com.

3. Transcriptional SASSI Regulation by Hormones and cAMP

The multitude of non-redundant isoforms within the PDE4 family results from alternative mRNA splicing and the use of different promoters within each of the four genes [29]. The structural differences between long and short isoforms underpin their differential regulation and, subsequently, the ability of cells to adapt to both short and long-term environmental stimuli [30]. Early work on SASSI revealed that they are subject to hormonal regulation: Sertoli cells treated with follicle-stimulating hormone had increased mRNA levels of rat *PDE4D1* and *PDE4D2* compared to unstimulated cells [20]. Characterization of the intronic promoter of PDE4D1/PDE4D2 confirmed its inducibility by hormonal stimulation, revealing further evidence for the versatility of the *PDE4D* gene [31]. In rat thyroid cells, *PDE4D1* mRNA was shown to be upregulated in response to long-term treatment with thyroid-stimulating hormone [23]. *PDE4D1* mRNA was also observed to change levels in Sertoli cells depending on their developmental state, with older, quiescent cells expressing more *PDE4D1* mRNA than both younger cells and terminally differentiated ones [32]. Interestingly, differences in SASSI expression have also been recorded in pregnancy: *PDE4B2* mRNA levels were reported to be higher in the myometria of pregnant women compared to non-pregnant women [33]. Treatment of cultured human myometrial cells with cAMP-elevating compounds induced specific upregulation

of both PDE4B2 and PDE4D1, indicating that their expression is influenced by hormones known to fluctuate during pregnancy [34]. Notably, preterm birth following infection has been linked to contractions induced by cytokine activity with a concomitant increase in PDE4 SASSI, highlighting the role of these isoforms in inflammation, as discussed in the Inflammation section below [35].

4. Differential Regulation of Short and Supershort Isoforms by ERK2

Unlike long PDE4 isoforms, which are inhibited by ERK2 phosphorylation, SASSI PDE4B2 and PDE4D1 are activated [11,36]. Interestingly, the supershort PDE4D2 has shown weak inhibition consequent to ERK2 phosphorylation: this has been attributed to its truncated UCR2, indicating that it is UCR2 that dictates the different outcomes of the conserved serine phosphorylation in the catalytic domain of SASSI [11]. At the time of writing, there are no published observations on the effect of ERK2 phosphorylation on PDE4B5 and PDE4D10 activity. It is, therefore, tempting to speculate that the effect would be similar to that on PDE4D2, as the aforementioned are supershort PDE4s [26–28]. Owing to its truncated catalytic domain and unique structure, the dead-short PDE4A7 does not seem to be regulated by ERK2 [11,15].

5. SASSI Influence Disease Progression

5.1 Inflammation

The fine-tuning of the inflammatory response would be impossible without the regulation of cAMP synthesis, localization, and degradation. Ever since the discovery of PDE roles in the cAMP pathway and, consequently, in the innate and adaptive immune responses, the topic continues to be an active research subject. Alongside the growing understanding of PDE biochemistry, studies on the signaling pathways targeted by asthma therapies have demonstrated the potential of PDE inhibitors as anti-inflammatory agents [37]. The interplay between the cAMP and inflammatory pathways has been extensively reviewed elsewhere [38]. Briefly, as a function of PDE inhibition, increased intracellular levels of cAMP lead to reduced cytokine release and decreased immune cell recruitment and activation, and vice versa. For the purpose of this review, the focus will be on the current knowledge of PDE4 SASSI as drivers of damaging, auto-inflammatory signaling in disease and injury.

Some of the first evidence that PDE4 SASSI are differentially regulated at different stages of immune cell activation has been obtained through the treatment of human monocytes with cAMP-elevating compounds. Increased *PDE4B2* mRNA expression in Mono Mac 6 cells has been reported within 1 hour of treatment with dibutyryl-cAMP. Expression of PDE4B2 mRNA was shown to peak after 3 hours of treatment, whereas PDE4D1 mRNA expression peaked between 2 and 8 hours of treatment. *PDE4B2* mRNA levels were shown to be decreased after 4 hours, and both SASSI mRNA were reported to return to basal levels at 24 hours. Protein expression was shown to follow a similar pattern: peak expression was observed between 3 and 5 hours of treatment with a gradual decrease to basal levels at 24 hours [39]. Further studies on Jurkat T cells have demonstrated that PDE4B2, PDE4D1, and PDE4D2 are among the very first PDE4 isoforms to be upregulated upon cAMP elevation [40]. Together, these findings demonstrate that the upregulation of SASSI is a compensatory response to increased cAMP levels.

In a study aiming to elucidate the exact role of PDE4 in T-cell activation, fully activated T cells (those with T-cell receptors (TCR) and CD28 receptors co-stimulated) were shown to recruit SASSI PDE4B2, PDE4D1, and PDE4D2 together with β-arrestin to lipid rafts on the cell membrane [41]. In an incompletely active state (upon TCR-only stimulation), steady cAMP production, which inhibits inflammatory signaling, was observed. On the contrary, cAMP levels in fully activated T cells were reported to be reduced. These findings led to a model in which the co-stimulation of TCR and CD28 induces recruitment of PDE4 and β-arrestin to lipid rafts where PDE4 activity reduces cAMP levels to enable maximal T-cell activation, highlighting the essential role of PDE4 SASSI in inflammation [41]. Expectedly, short-interference RNA (siRNA) knockdown of PDE4B and PDE4D in stimulated T cells has been reported to reduce their proliferation and cytokine production. Notably, the PDE4D-targeting siRNA was demonstrated to reduce T-cell proliferation to a similar extent as the pan-PDE4-targeting siRNA, suggesting that the PDE4D subfamily has the predominant role in T-cell activation [42]. On the other hand, PDE4B has been identified as a major regulator of cAMP in monocytes/macrophages, as discussed in the context of the disease in the following sections. A more detailed overview of the role of PDE4 in inflammatory processes exists [43].

5.2 Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by the progressive myelin sheath degradation of neuronal axons. Symptoms may be episodic or progressive and include cognitive impairment, fatigue, and muscle weakness [44]. It is estimated to affect 2.8 million people worldwide, with a mean age of diagnosis of 32 and female patients being twice as likely to live with MS compared to males [45]. Pathologically, MS results from myelin-reactive T- and B-cell infiltration of the CNS, which creates an inflammatory environment and drives neural degeneration. Current therapies aim to reduce inflammation, but curative treatment remains an unmet clinical need [44]. Studies on rat MS models have shown that *PDE4B2* mRNA is upregulated in microglia residing near brain vessels, indicating the isoform's involvement in disease progression [46].

In vivo studies have reported that PDE4B2 is, in fact, the only PDE4 upregulated in MS, and, importantly, it significantly correlates with disease score and the expression of some inflammatory markers [47]. The PDE4B inhibitor, A33, has been recently reported to reduce neuroinflammation by suppressing nitric oxide production in both human and murine macrophages, as well as lowering the levels of pathogenic T cells at the peak of disease in a mouse MS model [48]. Notably, CRISPR-*Cas9* knockdown of PDE4D1 and PDE4D6 in primary mouse oligodendrocyte precursor cells was shown to increase myelin basic protein levels, a marker of oligodendrocyte differentiation and remyelination [48]. Collectively, these findings highlight the potential of PDE4B and PDE4D SASSI as pharmacological targets in MS.

5.3 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a decline in memory and cognition which lead to behavioral changes and deterioration in speech, orientation, and motoric function. It is responsible for up to 80% of dementia cases, and its curative treatment remains an unmet clinical need [49]. Studies on mouse models of AD have shown that the administration of small molecule PDE4 inhibitors, including rolipram and roflumilast, improves cognition and reduces memory loss [50,51]. Importantly, PDE4 inhibition has also been demonstrated to significantly improve the cognitive performance of healthy young and older adults [52,53]. These findings strongly indicate that the PDE4 family of phosphodiesterases is a relevant clinical target for AD treatment.

In vivo studies on mouse AD models have shown that PDE4D but not PDE4B inhibition enhances cognitive capabilities [54]. What is more, an investigation into PDE4D regulation changes in AD has uncovered a significant association between PDE4D1 expression and cognitive impairment: in temporal lobe AD patient samples, increased PDE4D1 positively correlates with higher plaque pathology [55]. Together with a study proposing that PDE4D1 has a role in neuronal plasticity, these experimental outcomes have led to the conclusion that in AD, increased PDE4D1 expression is implicated in decreased neuronal firing rates and impaired memory formation, making it an attractive AD drug target [55,56].

5.4 Traumatic Brain Injury

Traumatic brain injury (TBI) resulting from road traffic injuries, sports concussions, and other accidents is estimated to affect around 69 million people worldwide each year, making it the biggest contributor to trauma-related disability and death [57]. The response to TBI develops within minutes and may result in neuronal apoptosis, further exacerbated by pro-inflammatory cytokine signaling, which reduces cAMP in microglia. Rolipram treatment of TBI rat models prior to injury has been demonstrated to restore cAMP levels, downregulate pro-inflammatory signaling, and reduce neuronal apoptosis following TBI [58]. SASSI PDE4B2 (in neuronal dendrites) and PDE4D2 (in ipsilateral cortex cells) have been identified as undergoing significant upregulation as early as 30 minutes post-TBI in rat models and maintaining significantly higher levels compared to control animals until 24 hours post-injury [59]. In addition, a study on the effect of pro-inflammatory cytokines on post-TBI microglial cAMP levels has revealed significantly increased protein levels of both PDE4B2 and PDE4A1 within 30 minutes of inflammatory stimulation. Knockdown of PDE4B2 using siRNA has demonstrated its essential role in microglial activation through increased cAMP degradation [60]. Localization studies have indicated that PDE4B2 is expressed in both neurons and infiltrating leukocytes near the contusion site, whereas PDE4D is predominantly found in immune cells but not neurons, suggesting that PDE4B2 may have a role in both inflammation and memory deficits following TBI [61]. Notably, treatment of TBI rat models with a PDE4B-specific inhibitor has been reported to reduce inflammation, neuronal apoptosis, and memory deficits, thus contributing to the growing list of evidence in support of the clinical relevance of PDE4 SASSI in neuroinflammation [62].

5.5 Cancer

Aberrant intracellular signaling underpins carcinogenesis, and, expectedly, altered cAMP levels resulting from changes in PDE4 expression are implicated in malignancy. *In vivo* studies have demonstrated that PDE4A1 overexpression in murine brain tumor xenografts correlates with lower cAMP levels in tumor cells and significantly decreased cancer cell doubling times. Notably, when combined with first-line therapeutics, rolipram has been shown to improve the survival of mice bearing intracranial tumors [63]. Further work on optic pathway glioma mouse models has affirmed the importance of PDE4A1 in brain carcinogenesis, as cortical PDE4A1 overexpression has been shown to lead to the formation of tumors similar to those observed in patients [64].

In a colorectal carcinoma (CRC) cell line, PDE4B2 has been observed to be upregulated by the mutant Kirsten rat sarcoma (KRAS) oncoprotein, which has been shown to induce acinar structure disruption in three-dimensional (3D) cultures through Ak strain transforming (AKT) phosphorylation and activation. AKT phosphorylation has been reported to be ameliorated by rolipram. Notably, *PDE4B2* knockdown has been found to re-establish healthy luminal apoptosis in 3D cultures [65]. These findings have been further corroborated by work on KRAS-mutant mouse models, in which PDE4B2 was demonstrated to enhance tumor growth, while the small molecule PDE4 inhibitor apremilast reduced tumor volume [66]. Altogether, these data strongly suggest that PDE4B2 may be a viable therapeutic target in CRC.

In a study focused on PDE4D oncogenicity, PDE4D shRNA treatment of a melanoma cell line stably expressing exogenous PDE4D2 was reported to exhibit significantly less tumor cell apoptosis compared to control melanoma cells undergoing the same treatment: an indication that PDE4D2 prevents tumor cell death. Notably, upon ectopic PDE4D2 expression, increased proliferation has been observed in both melanoma and gastric cancer cells in support of the hypothesis that PDE4D2 dysregulation is implicated in tumor progression [67].

5.6 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is an irreversible systemic condition affecting more than 200 million people globally and is the third leading cause of death worldwide. Risk factors include genetic predisposition, tobacco smoking, infections, malnutrition, and air pollution. Patients may experience dyspnoea, coughing, and wheezing, which worsen if not managed, as curative treatment is yet unavailable [68].

Progressing COPD is characterized by a significant increase in $CD8^+$ T-cell, neutrophil, and macrophage numbers in the inflammatory infiltrate of the lung epithelium, contributing to lung function decline [69]. Analysis of PDE4 mRNA levels in neutrophils and monocytes of healthy donors has revealed that PDE4B2 is the predominantly expressed isoform: while neutrophils have a constitutively high PDE4B2 expression, it is inducible in monocytes. Notably, compared to other sites, including the lung, brain, and spleen, *PDE4B2* cDNA levels have been reported to be the highest in leukocytes, indicating the potential of this short isoform as an anti-inflammatory drug target [70]. What is more, tolerance to roflumilast in patients with exacerbated COPD, driven by non-typeable *Haemophilus influenzae* (NTHi), has been demonstrated to result from the upregulation of PDE4B2 [71]. The synergy between roflumilast and NTHi has been reported to drive the increase in PDE4B2 expression, and PDE4B2 has been suggested to have two roles in the inflammation driving COPD: as a hydrolase and as an adaptor protein in the inhibitor of nuclear factor kappa B pathway driving chemokine upregulation [71]. Additionally, increased levels of monocyte *PDE4B2* transcripts have been reported in smokers compared to non-smokers [72], further emphasizing the importance of this isoform in COPD and its potential as a drug target.

6. Conclusions

The discovery of the *dnc* gene and its role in *D. melanogaster* behavior instigated extensive research into rodent and human homologs, leading to the characterization of the PDE4 family of phosphodiesterases. Their essential role in compartmentalized intracellular signaling means that even minute changes in expression or regulation induce abnormal cAMP effector activity and pathology. While full-length PDE4 isoforms have long been in the spotlight as drug targets for the treatment of auto-inflammatory diseases, targeting PDE4 SASSI remains largely uncharted. The present review has summarised the current knowledge of PDE4 SASSI in pathology and has thus highlighted their potential as pharmacological targets in several diseases, for most of which there is no curative treatment available.

Isoform-selective inhibition of PDE4 remains an obstacle, as clinically approved small molecules, including roflumilast, apremilast, and crisaborole, rely on binding the catalytic pocket of cAMP hydrolysis, which is highly conserved across isoforms within each PDE4 subfamily. Non-selective binding to long PDE4 isoforms causes dizziness, nausea, emesis, and gastrointestinal side effects in patients, making PDE4 inhibitors second-line treatment at best [16]. While some selectivity for long isoforms has been achieved with the allosteric inhibitor BPN14770 which has been reported to have higher potency against PDE4D3 and PDE4D7 than against PDE4D2 [73], most molecules in development and in trials either inhibit all PDE4 isoforms or exhibit a higher selectivity for one of the four subfamilies [74]. The major structural difference between long and short isoforms, namely the lack of UCR1 in SASSI and their subsequent inability to form dimers, could be utilized in the development of isoform-selective PDE4 inhibitors. Therapeutics targeting unique domains of PDE4 SASSI have the potential to overcome the long-standing challenge of isoform-selective PDE4 inhibition to ultimately improve patient quality of life.

Author Contributions

EK and GSB jointly conceptualized, searched available literature, and wrote the original draft. EK made the figures. GSB edited the final draft. EK made amendments following the review. Both authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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