



PDE4 subtypes in cancer

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Abstract

Cyclic nucleotide phosphodiesterases (PDE) break down cyclic nucleotides such as cAMP and cGMP, reducing the signaling of these important intracellular second messengers. Several unique families of phosphodiesterases exist, and certain families are clinically important modulators of vasodilation. In the current work, we have summarized the body of literature that describes an emerging role for the PDE4 subfamily of phosphodiesterases in malignancy. We have systematically investigated PDE4A, PDE4B, PDE4C, and PDE4D isoforms and found evidence associating them with several cancer types including hematologic malignancies and lung cancers, among others. In this review, we compare the evidence examining the functional role of each PDE4 subtype across malignancies, looking for common signaling themes, signaling pathways, and establishing the case for PDE4 subtypes as a potential therapeutic target for cancer treatment.

Introduction

There are over 20 cyclic nucleotides phosphodiesterases that are known to regulate cAMP and cGMP levels. Cyclic nucleotide phosphodiesterases work by binding to cyclic nucleotides and degrading them into non-cyclical monophosphates. The cyclic nucleotide phosphodiesterases are subdivided into 11 families, each of which contains several unique isoforms. PDE4, PDE7, and PDE8 are specific for hydrolysis of cAMP. PDE5, PDE6, and PDE9 are specific for hydrolysis of cGMP. PDE1, PDE2, PDE3, PDE10, and PDE11 are nonspecific and will hydrolyze both cAMP and cGMP [1]. The history of successfully targeting specific PDE isoforms is variable, and inhibitors for PDE3, PDE4, and PDE5 are presently available in the United States. PDE3 inhibitors are used for heart failure and peripheral artery disease, owing to their vasodilatory properties [2].

PDE5 inhibitors are used for pulmonary hypertension and erectile dysfunction, also resulting from tissue-specific vasodilatory properties [3]. PDE4 inhibitors, on the other hand, are potent inhibitors of inflammation, and they have been approved for the treatment of inflammatory diseases ranging from arthritis to chronic obstructive pulmonary disease. In addition, PDE4 inhibitors are approved as oral or topical treatments for psoriasis and atopic dermatitis, respectively. The main challenge with PDE4 inhibitors is their very narrow therapeutic index and limiting gastrointestinal tolerability due to nausea, emesis, and diarrhea [4, 5].

Because PDE4 is such an appealing clinical target for many additional non-dermatologic indications, additional research has continued to pursue strategies to widen the therapeutic index. Nearly 10 years ago, it was found that small-molecule inhibitors that only partially inhibit the activity of specific PDE4 isozymes have reduced adverse effect profiles with sustained clinical potency [6]. PDE4 enzymes are generally categorized into four subtypes (PDE4A, PDE4B, PDE4C, and PDE4D), and promising therapeutics are currently in development for neurologic disorders that target PDE4B and PDE4D [7–9]. The renewed interest in subtype-specific PDE4 inhibitors warrants a comprehensive investigation into the specific PDE4 subtypes and their potential role in inflammatory pathologies. The aim of the current study is to provide a review of the current literature on the four subtypes of PDE4 and their role in cancer, specifically.

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Results

PDE4A in cancer

Our search results produced 17 total studies investigating the PDE4A subtype with nine of these studies examining PDE4A specifically and eight studies examining PDE4A in conjunction with at least one other PDE4 subtype. These studies were further categorized by specific type of malignancy with the following results: hematologic ($n = 7$), CNS and adrenocortical tumors ($n = 6$), lung cancer ($n = 2$), liver cancer ($n = 1$), oral cancer ($n = 1$), and breast cancer ($n = 1$). One study addressed the role of PDE4A in the context of multiple malignancies thus a total of $n = 19$.

CNS tumors

Five studies examined the association of PDE4A with various CNS tumors. Four studies looked at the expression levels of PDE4A in various CNS tumors. PDE4A was found to have low expression in normal pituitary tissue [10] and overexpression in medulloblastoma, glioblastoma, glioma, and somatotroph, lactotroph, corticotroph, and nonfunctioning gonadotroph adenoma cells [10–13]. No studies reported the decreased expression of PDE4 in malignant CNS tissues.

With a reported overexpression of PDE4A in CNS tumors, two studies sought to determine whether or not this increased expression resulted in increased PDE4 activity. It was found that PDE4A overexpression resulted in significantly shortened doubling times in medulloblastoma and glioblastoma cells [11] as well as hypercellular lesions with human neurofibromatosis 1-associated glioma features [12], suggesting a role for the elevation of PDE4A in tumor proliferation.

Two studies examined the interaction of PDE4A with the aryl hydrocarbon receptor-interacting protein (AIP) gene and the associated effects of disrupting this interaction. Although the function of AIP is not well described, it is thought to act as a tumor suppressor involved in the regulation of cell proliferation. Naturally occurring oncogenic variants in AIP that disrupted the PDE4A-AIP interaction played a significant role in pituitary tumorigenesis [10]. This disruption was also observed in the somatotropinoma and adrenocortical carcinoma tumor cells of a MEN1- and p53-negative mother–daughter pair with acromegaly due to somatotropinoma [14]. In these patients, a functional mutation blocking the AIP protein from interacting with PDE4A was identified in the tumoral DNA of the daughter's somatotropinoma and the mother's adrenocortical carcinoma.

Hematologic malignancies

Association with hematologic malignancies constitutes the most common area of research for PDE4A in cancer. Six

studies investigated PDE4A expression in hematologic malignancies including B-cell chronic lymphocytic leukemia (B-CLL), chronic myelomonocytic leukemia (CML), myelodysplastic syndrome, T cell leukemia, essential thrombocytopenia, polycythemia vera, and primary myelofibrosis. Of the six studies, one saw a decrease in PDE4A expression in CD34+ granulocytes taken from patients with essential thrombocytopenia, polycythemia vera, and primary myelofibrosis [15]. In contrast, the other five studies found a distinct upregulation of PDE4A expression in chronic lymphocytic leukemia, myelodysplastic syndrome, and T-leukemic cell lines [16–20]. In addition, it was found that PDE4A is the most abundantly expressed PDE subtype in B-lymphoblastoid cell lines [19] and T-leukemic cell lines [19, 20]. Interestingly, quiescent isolated human peripheral blood lymphocytes express mRNA for PDE4B as the principal transcript and it is only after mitogenic stimulation with phytohemagglutinin, a known initiator of mitosis, that mRNA for PDE4A is induced [19], suggesting that PDE4A upregulation may not be an early event in carcinogenesis.

One study examining the functional role of PDE4A in hematologic malignancy investigated the apoptotic pathway following exposure to Yessotoxin, a potent inducer of apoptosis in human leukemic cell lines. They found that PDE4A is required only for non-apoptotic cell death, driven primarily by autophagy, following Yessotoxin exposure [21]. Another study found that lower PDE4A expression is indicative of a response to a hypomethylating agent in patients with myelodysplastic syndrome and CML [18]. Taken together, these data suggest that PDE4 expression may be associated with resistance to cytotoxic agents.

Lung, liver, and oral cancers

Two studies examined PDE4A in lung cancer and both studies found PDE4A expression to be upregulated in various lung cancer cell lines. One study found that PDE4A expression induces epithelial–mesenchymal transition, a critical event in the pathogenesis of organ fibrosis and cancer, in alveolar epithelial type 2 cells following stimulation with transforming growth factor (TGF)- β 1. Both PDE4A mRNA and protein expression is upregulated following (TGF)- β 1 stimulation, resulting in significant loss of E-cadherin [22], a process that has been linked to metastasis. Another study demonstrated increased PDE4A expression during hypoxia by hypoxia-inducible factors across a panel of lung cancer cell lines. In addition, this study linked PDE4A to tumor cell proliferation and colony formation and showed that PDE4A knockdown can reduce VEGF secretion and have antitumor effects in lung cancer xenografts [23]. A singular study in hepatocellular carcinoma identified a role for PDE4 in driving

epithelial–mesenchymal transition by increasing N-cadherin and vimentin while simultaneously decreasing E-cadherin [24]. Finally, one study showed that PDE4A is down-regulated in mice treated with black raspberry chemopreventive agent [25]. These highly mechanistic studies support a role for PDE4A in tumorigenesis and metastasis.

Breast cancers

There is presently only a single study linking PDE4A to breast cancer. However, this study deserves mention because it linked lower expression of PDE4A to a decrease in progression-free and overall survival. This study, in stark contrast to other studies investigating PDE4A, shows that an increase in PDE4A expression is associated with better outcomes. This study found that PDE4A expression was elevated in estrogen receptor (ER) positive and progesterone receptor positive breast cancer [26], two factors correlated with improved prognosis. More studies are warranted to confirm these findings in breast cancer.

PDE4B in cancer

Our search identified 34 studies investigating the PDE4B subtype and its role in cancer. The role of PDE4B in neoplasia was investigated across multiple reports in hematological ($n = 16$), colon ($n = 8$), and lung ($n = 2$). Singular reports examined the role of PDE4B in cancers of the liver, kidney, oral cavity, breast, CNS, endometrium, skin, and prostate cancer.

Hematologic malignancies

Of all the subtypes and malignancies, PDE4B and its role in hematologic malignancies comprises the largest body of literature on PDE4 and cancer with a total of 16 research studies. PDE4B has been examined in the setting of various hematologic neoplasias including diffuse large B-cell lymphoma (DLBCL), acute lymphoblastic leukemia (ALL), chronic lymphoblastic leukemia (CLL), and multiple myeloma (MM).

Regardless of the specific malignancy, the agreement across multiple studies was that PDE4B expression was found to be elevated in hematologic malignancies. Increased PDE4B was observed in DLBCL [27–29], CD4+ lymphoid cancer cells [30], CLL [17] and B-lymphoblastoid cells [19, 16]. One study found that a single nucleotide polymorphism (SNP) (rs12142375) linked to ALL risk is also an important regulator of PDE4B expression [31], thus providing a potential genetic target between PDE4B and hematologic malignancies.

In addition, multiple studies found an inducible upregulation of PDE4B expression in hematologic malignancies

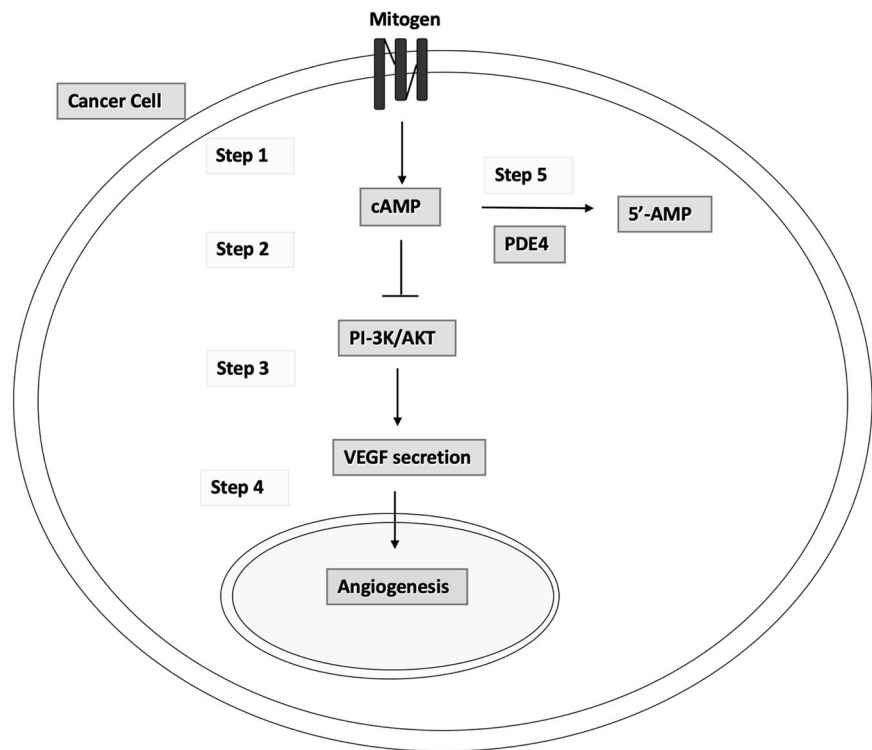
[19, 29, 30, 32]. PDE4B was readily upregulated in activated human peripheral blood mononuclear cells (PBMC) following stimulation with IL-2, the primary growth factor for promoting survival and proliferation of activated T cells. This observation, combined with the finding that PDE4B is expressed in CD4+ lymphoid cancer cells, but not primary CD4+ T cells, suggests a role for PDE4B in the survival and proliferation of cancer cells [30].

It should be mentioned that PDE4B levels also appear to increase in the presence of elevated cAMP as part of a negative feedback loop. PDE4B was upregulated in T-leukemic and B-lymphoblastoid cells after mitogenic stimulation with 1-methyl-3-isobutylxanthine (IBMX) and the cAMP analog, dibutyryl cAMP (dBcAMP) [19]. Likewise, PDE4B was upregulated in MM cells following treatment with HENECA, a mitogenic A_{2A} receptor agonist, and rolipram [29]. Rolipram is a nonselective PDE4 inhibitor. When rolipram alone was used to treat CLL cells in another study, a marked upregulation of PDE4B occurred [32], suggesting that the upregulation of PDE4B is in response to the pathologic accumulation of cAMP in lymphoid cancer cells following rolipram treatment.

A number of studies also examined the associated effects of PDE4B expression in hematologic malignancies. Increased PDE4B expression was correlated with poorer outcomes in primary DLBCL, and an association was found between the inhibition of phosphatidylinositol 3-kinase/AKT (PI3K/AKT) pathway and cAMP-mediated apoptosis [27]. Similarly, increased PDE4B expression was associated with significantly higher microvessel density, which is an abnormal vascular network critical for tumor metabolism and metastatic potential. This was associated with poorer outcomes in DLBCL [28]. This association was reinforced when a Myc-induced lymphomagenesis in mice bearing a PDE4B-null background showed lower microvessel density compared with PDE4B wild-type mice [28]. These responses were attributed to modulation of vascular endothelial growth factor (VEGF) via PDE4B-dependent downregulation of cAMP and subsequent effects on the PI3K/AKT pathway [28]. Thus, the PDE4B/cAMP/PI3K/AKT/VEGF pathway may play an important role in the pathogenesis of hematologic malignancies (Fig. 1).

Four studies examined the clinical applications of these findings in the context of PDE4B, hematologic malignancies, and pharmacologic resistance. Using gene set enrichment analysis, overexpression of PDE4B in DLBCL was found to impinge on the cAMP inhibitory activities of the AKT/mTOR pathway and define glucocorticoid (GC) resistance. Furthermore, inhibition of PDE4 in a xenograft model of human lymphoma restored GC sensitivity [33]. This is supported by an in vitro study demonstrating that miR-124, an established anti-tumorigenic microRNA, influences GC-induced apoptosis in DLBCL by directly

Fig. 1 A model for the role of PDE4 in the promotion of angiogenesis. PDE4 upregulation is a late event in many cancer cells preceded by the following steps: (1) A mitogen activates cAMP in cancer cells. (2) cAMP inhibits the PI3K/AKT pathway. (3) Inhibition of the PI3K/AKT pathway results in decreased VEGF secretion. (4) Decreased VEGF leads to decreased angiogenesis. (5) Decreased angiogenesis leads to compensatory upregulation of PDE4 through HIF, and subsequent increased activity of the PI3K/AKT pathways and enhanced VEGF secretion and angiogenesis.



targeting PDE4B and stable expression of miR-124 in DLBCL cell lines diminishes PDE4B expression [34]. Using a genome-wide approach, an association was identified between PDE4B SNPs and risk of relapse in patients with childhood ALL [35]. Lastly, genetic inhibition of PDE4B improved the efficacy of SYK inhibitors through control of cAMP-modulated phosphorylation and activity of the tyrosine kinase SYK [36].

Colon cancers

Our systematic search identified eight studies that associated the PDE4B subtype with colon cancer. With the oldest study published in 2011, this collection represents some of the most recent literature of this scoping review. There may be a connection between the KRAS–PDE4B interaction and the development and survival of colonic cancer cells. One study showed that PDE4B expression is upregulated by oncogenic KRAS [37]. The same study analyzed public datasets and showed the higher expression of PDE4B in tumor samples from colorectal cancer patients when compared with those from healthy control. In addition, increased expression of PDE4B mRNA was found to be correlated with relapsed colorectal cancer in this public data subset [37]. In biopsies obtained from patients with and without colorectal neoplasia, both PDE activity and expression were lower overall in colorectal neoplasia while real-time qPCR analysis showed overexpression of the subtype PDE4B, suggesting that PDE4B is selectively

overexpressed as a malfunctioning protein in non-neoplastic appearing colonic mucosa from colorectal neoplasia patients [38]. Indeed, PDE4B RNA appears to be one increased in both colonic adenomas and adjacent normal colonic tissues, and a protective role has been hypothesized for it in the adjacent normal tissues [39]. Additional studies also support the claim that PDE4B is signaling downstream of mutant KRAS in vitro [40, 41], and one study proved novel compounds capable of PDE4B-specific docking and inhibition to be superior to traditional chemotherapeutics such as doxorubicin in cell based assays [42]. Additional work suggested that PDE4B is also downstream of the Myc oncogene, and PDE4B works to suppress cAMP-mediated inhibition of the AKT/mTOR pathway in colon cancer models [43]. A singular study examined the development of colon cancer and the effects of diet on carcinogenesis. The protein level of PDE4B was upregulated while miR-26b, the microRNA associated with PDE4B, was downregulated in rats fed a chemoprotective diet [44]. These findings suggest that PDE4B may play a role in colon cancer, particularly in cases with KRAS activating mutations.

Other cancers

Singular and dual reports of PDE4B's involvement in various other malignancies included cancers of the lung ($n = 2$), liver ($n = 1$), kidneys ($n = 1$), oral cavity ($n = 1$), prostate ($n = 1$), breast ($n = 1$), CNS ($n = 1$), endometrium ($n = 1$), and skin ($n = 1$). Similar to the previously described

findings, PDE4B expression was found to be increased in non-small cell lung cancer tissues [45], human glioblastoma cell lines [13], and human gingiva-derived malignant melanoma cell lines [46]. Studies examining the functional properties of PDE4B also concurred with this oncogenic role of PDE4B. PDE4B was associated with anti-apoptotic and metastatic effects in kidney cancer [47] and endometrial cancer [48], and cell growth regulation through cAMP in malignant melanoma [46]. Further, two additional studies highlight in vitro findings that specific PDE4B inhibition is both cytotoxic in A549 lung cancer cells [49] and growth inhibitory in oral cancer cells [50].

In contrast to these studies, PDE4B was found to be downregulated in castration-resistant prostate cancer and advanced prostate cancer [51]. Taken alone, this seems like an outlier, but considered in conjunction with the singular study describing the downregulation of PDE4A in breast cancer [26], it should be noted that PDE4 subtypes may play different roles in hormonally regulated cancers and this is an interesting area for future investigation.

PDE4C in cancer

Our search identified seven studies pertaining to PDE4C and cancer. None of these studies examined the role of PDE4C in the same malignancy. The studies examined malignancies of the blood [18], skin [52], central nervous system [53], lung [45], thyroid [54], and one study focused on malignancies associated with p53 mutations in particular [55].

In high-grade glioma samples, there was hypermethylation of PDE4C promoter sites and hypomethylation in low-grade glioma [53]. This relationship is important to note as it contrasts the overexpression of PDE4A seen in CNS tumors. In other malignancies, however, PDE4C expression follows the more common trend of overexpression in patients with myelodysplastic syndrome [18] and thyroid adenomas [54]. PDE4C was also identified as a novel target gene of mutated transcription factor p53, potentially linking this subtype to a wide range of p53-associated malignancies [55].

PDE4D in cancer

Our search results produced 44 studies examining PDE4D and its role in cancer making PDE4D the most examined PDE4 subtype in this study with a wide distribution of focus covering hematologic ($n = 4$), lung ($n = 5$), prostate ($n = 7$), skin ($n = 4$), head and neck ($n = 6$), CNS ($n = 3$), colon and gastric ($n = 6$), breast ($n = 4$), bladder ($n = 1$), pancreatic ($n = 1$), and ovarian ($n = 1$) malignancies. One study also examined PDE4D across solid tumors in general.

Hematologic malignancies

Four studies focused on PDE4D and hematologic malignancies. Two of these studies examined baseline PDE4D expression in hematologic malignancy. Interestingly, a 30-fold decrease in PDE4D mRNA was observed in cells taken from patients with chronic lymphocytic leukemia compared with PBMC from healthy adults [56] while PDE4D was one of the most abundantly expressed PDEs in Jurkat T-leukemic cell lines [20] suggesting a discrepancy in expression between hematologic malignancies.

Two studies also examined PDE4D expression following various mitogenic and pharmacologic stimulation. An increase in PDE4D mRNA expression was seen in human peripheral blood cells following stimulation with phytohemagglutinin (a mitogen used to trigger T-lymphocyte cell division) and in B-lymphoblastoid cell lines following incubation with dBcAMP and IBMX to mimic phytohemagglutinin's mitogenic effects [19]. In addition, PDE4D expression was upregulated in ALL cells but not B-CLL cells following increased activation of adenylyl cyclase using forskolin [32]. Together, these findings may suggest an important role for PDE4D in rapid cell division.

Lung cancers

PDE4D appears to play an important role in the development and progression of lung cancer. PDE4D expression was found to be regulated by the mutant form of serine/threonine kinase 11/liver kinase B1, a kinase present in lung cancer [57] and is positively correlated with mTOR expression [58]. Increased PDE4D expression was also noted in response to hypoxia in 8/10 lung cancer cell lines [23] and TGF-beta1 stimulation to mimic epithelial–mesenchymal transition, a critical event in the pathogenesis of organ fibrosis and cancer [22]. In addition, PDE4D expression was linked to tumor cell proliferation [23, 57], tumor cell differentiation [59], and loss of E-cadherin [22]. Together, these studies form a strong foundation connecting PDE4D to the both the development and progression of lung cancer making it a prime target for future investigations.

Prostate cancer

The seven studies produced by our search suggest a role for PDE4D in prostate cancer, but it appears that specific subtypes of PDE4D may play individual roles in prostate cancer. PDE4D7 was found to be highly expressed in androgen-sensitive prostate cancer cells and starkly downregulated in androgen-insensitive cells, connecting a low PDE4D7 expression with poorer outcomes [60, 61]. In addition, PDE4D7 is upregulated in primary human prostate cancer while PDE4D5 and PDE4D9 are downregulated

[62, 63]. Studies that examined PDE4D in general found an overall overexpression in prostate cancer [64] and found that PDE4D inhibition lead to growth inhibition [64, 65], increased apoptosis, and decreased proliferation and migration of prostate cancer cells [65]. Two studies also examined promoter sites and found that the CpG site of PDE4D is differentially methylated in prostate cancer [66] and noted an increased promoter methylation of PDE4D5 [62] suggesting potential markers for disease staging and/or targeted treatment. Together, these studies show an upregulation of PDE4D in prostate cancer but also highlight the potential of specific PDE4D subtypes in classification, prognostication, and treatment of prostate cancer.

Skin cancers

PDE4D was found to be overexpressed in BRAF-mutated melanoma cell lines and this PDE4D overexpression promotes invasion through its interaction with focal adhesion kinase via the scaffolding protein RACK1 [67]. The PDE4D gene was also found to be negatively associated with survival in patients with metastatic melanoma lesions using gene expression profiling [68]. PDE4D also appears to play an important role mechanistically in the development of skin cancer by regulating cell growth through cAMP in human gingiva-derived malignant melanoma cell lines [46]. Interestingly, PDE4D expression was also found to be upregulated in melanocytes by MSH/cAMP/MITF pathway, leading to reduced melanocyte pigmentation [69]. Inhibition of PDE4D *in vivo*, likewise, resulted in an enhancement of skin pigmentation. However, the changes induced by topical inhibition of PDE4D in these mice did not exhibit melanocytic features.

Head and neck cancers

With a total of six studies, head and neck cancers are the third-most investigated malignancy with respect to PDE4D, although the role of PDE4D in the pregression of head and neck cancers is far from clear. In nasopharyngeal carcinoma, PDE4D expression was found to be overexpressed *in vitro* and *in vivo* via western blot [70]. Lentiviral knockdown of PDE4D also led to the downregulation of AKT signaling pathway via EGFR, resulting in cell cycle arrest in G0/G1 phase. Contrary to this work, chromosomal profiling of abnormalities using high-density SNP arrays in 101 patient samples with esophageal carcinoma at various stages showed a positive correlation between disease progression and recurrent loss of chromosome regions, resulting in disruption of single genes including PDE4D in losses from chromosome 5q [71]. However, the effects resulting from the disruption of PDE4D in these patients are not characterized. A separate whole genome SNP array of 23

primary esophageal adenocarcinoma samples identified 126 homozygous deletions that included known tumor suppressor genes such as CDKN2A (p16) and SMAD4, as well as gains in proto-oncogenes including MYC and BCL9, and this work also detected a homozygous deletion of PDE4D at 5q [72]. These findings in esophageal adenocarcinoma and nasopharyngeal carcinoma highlight the possible multiple roles of PDE4D in cancer progression. This is further supported by contradictory data about PDE4D SNPs and esophageal squamous cell carcinoma in genotype studies with individuals of diverse ancestries [73].

Analysis of miRNA expression on thyroid adenoma found direct repression of PDE4D mRNA by two miRNA at the 3'UTR region [74]. Downregulation of PDE4D miRNA may explain the low cAMP levels in thyroid adenoma, as well as their relatively benign characteristics. Similarly, an increase in PDE4D expression activity was found in 18 primary thyroid adenoma samples with mutant TSH receptor Gsa, while maintained in normal thyroid tissues [54].

CNS

Similar to PDE4A findings in CNS tumors, in which PDE4A overexpression resulted in increased growth of medulloblastoma and glioblastoma cells, expression of PDE4D was found to enhance Hedgehog signaling in medulloblastoma via direct interaction with Neuropilins and subsequent indirect inhibition of protein kinase A [75]. Inhibition of PDE4D in mouse allograft model led to the suppression of Hedgehog and the inhibition of medulloblastoma growth. Likewise, in glioblastoma, treatment with a novel PDE4D inhibitor, rolipram, led to cell cycle arrest and differentiation of glioblastoma derived cells [76]. This effect may be mediated via the upregulation of cAMP/CREB signaling pathway due to the loss of PDE4D activity. In addition, a study of DNA alterations 30 glioma patients via AP-PCR found that altered PDE4D was associated with high levels of chromosomal instability and in both primary and secondary glioblastoma [77]. The presence of PDE4D alterations were also associated more commonly with wild-type p53 and p16, suggesting that decreased cAMP concentration may play a role in tumor suppression mechanism of p53 and p16. However, the phenotypic traits of these PDE4D alterations were not characterized. Interestingly, these PDE4D alterations were not found to have a significant impact on patient survival.

Colon and gastric cancers

In colon cancer cell lines, an underexpression of a specific p53 induced microRNA, miR-129-5p, was found to cause an elevated expression of PDE4D, resulting in oncogenic

activity [78]. Treatment with miR-129-5p suppressed expression of PDE4D, leading to cAMP/BIM mediated colon cancer cell cycle arrest and apoptosis. Similarly, other studies found that, growth and survival in colon cancer cells are dependent on a PDE4D [79–81]. PDE4D expression has also been linked to co-expression with another novel oncogene, PIWIL1, in gastric cancer cells [82]. Interestingly, a loss of PDE4D in a colon adenocarcinoma cell line, SW480, demonstrated an acquired resistance against triapine, an anticancer iron-chelator belonging to the thiosemicarbazone class [83]. This chemotherapy resistance is associated with an upregulation of cAMP and Epac/Rap1 signaling pathway, which has a role in metastasis and metabolism in solid tumors, and it is in stark contrast another study that described increased sensitivity to doxorubicin in gastric cells with miR-494 expression and silencing of PDE4D [84]. Thus, PDE4D may be associated with both oncogenic and chemosensitivity functions in gastric and colon cancers.

Breast, bladder, and pancreatic cancers

In a large-scale exome-wide analysis of 8287 subjects for rare variants with minor allele frequency in women of African ancestry in the African American Breast Cancer Epidemiology and Risk Consortium [85], PDE4D was found to be associated with ER negative and progesterone, estrogen, and human epidermal growth factor receptor negative (triple negative) breast cancers, but does not show an overall increased risk for breast cancer. In a separate whole genome parallel sequencing of BRCA1 mutation positive breast cancer with either ER+ or ER-expression, recurrent homozygous deletion of PDE4D was found in the ER negative subset of BRCA1 positive breast cancers [86]. In addition, a PDE4D SNP was found to be associated with higher acini count per terminal duct lobular units, an established breast cancer risk factor, in a pooled analysis of 872 women [87] and a PDE4D-CCNB1 fusion gene was identified in two endometrial tumors [88]. PDE4D expression was also associated with CD177 co-expression and neutrophil recruitment into pancreatic cancers, corresponding with poor prognosis [89]. A more mechanistic study showed that PDE4D inhibition may provide a means to overcome tamoxifen resistance in ER positive breast cancer models [90]. Conversely, low PDE4D levels were correlated with poor prognosis in bladder cancer patients [91].

Discussion

Although there is some evidence that PDE4A may be involved in the regulation of tumor suppressor genes, the

more abundant theme is that PDE4A upregulation is a response to mitogenic stimulation in tumors and hematologic malignancies. In fact, in solid tumors, data suggest that PDE4A may be involved in VEGF-mediated angiogenesis and later epithelial–mesenchymal transition and metastasis.

There is a large body of literature supporting a role for PDE4B in hematologic malignancies. The nonspecific PDE4 inhibitor roflumilast has entered early phase I clinical trials for B-cell malignancies [92]. The purported mechanism for these agents is through renewed suppression of AKT-mediated pathways by cAMP. There was also substantial evidence linking PDE4B upregulation to cancers of the colon, perhaps as a downstream signaling effect of KRAS mutation.

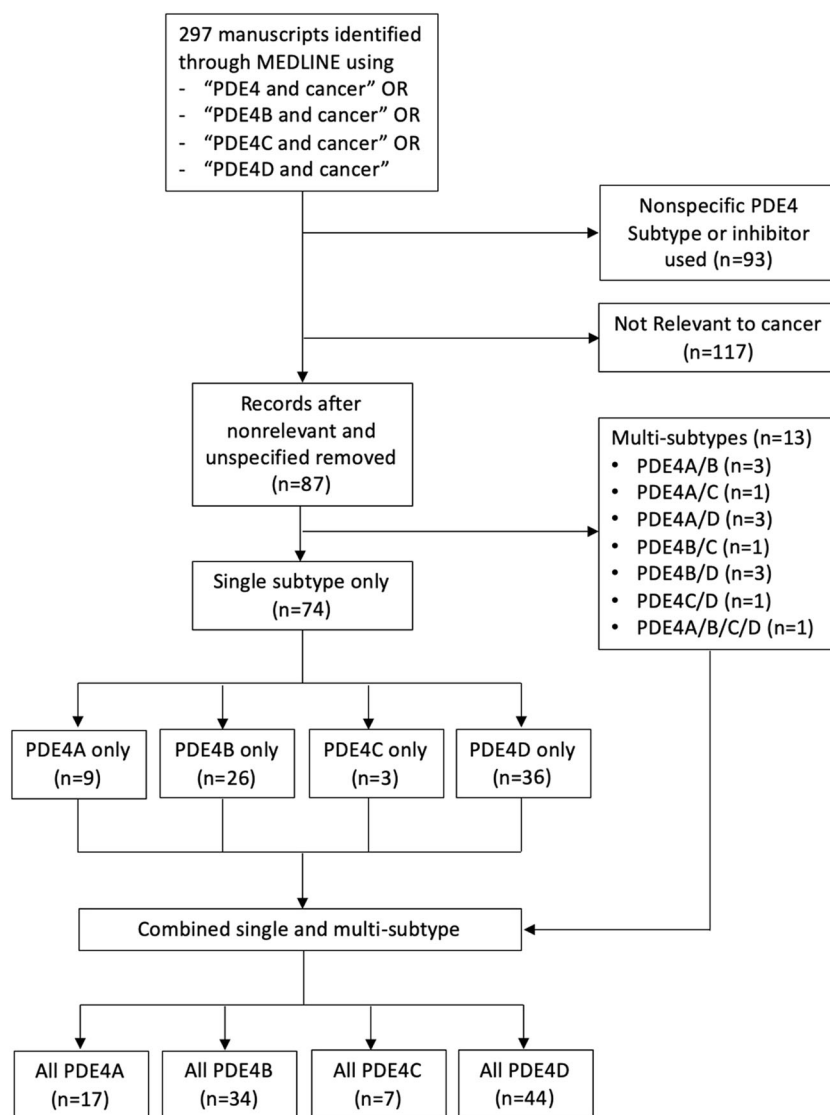
The literature supporting a role for PDE4C in cancer is both scant and contradictory. Given the lack of clinically available tools for targeting PDE4C, and the paucity of data supporting a role for PDE4C-mediated oncogenesis, this seems like the PDE4 subtype that is least worth pursuing for future studies.

PDE4D is the most heavily studied of all the PDE4 subtypes. While there is conflicting data about the role of PDE4D in hematologic malignancies, the data are more clear with PDE4D in solid tumors. There is strong evidence that PDE4D contributed to tumor progression in lung, CNS, and skin cancers, while specific isoforms of PDE4D play opposing roles in prostate cancer.

While the focus of this work was to provide a scoping review of the published literature on PDE4 subtypes in cancer, it is worth briefly discussing that we excluded 93 works for their use of nonspecific PDE4 subtype inhibitors (Fig. 2) and these works may include helpful studies that act through many of the pathways described herein. Indeed, combination therapies of nonselective PDE4 inhibitors with other antineoplastic agents have been tested in wide variety of pre-clinical cancer models to target the pathways described here. Examples include the use of rolipram with bevacizumab, an inhibitor of VEGF, in glioblastoma models [93, 94] and roflumilast with idelalisib, a PI3K inhibitor, in DLBCL and CLL [95]. Like the many other targeted therapies that may be used successfully in combination, the synergistic effects of PDE4 inhibition with concomitant inhibition of other mitogen-stimulated pathways should not be overlooked as a therapeutic strategy.

One concern with the use of PDE4 inhibitors for the treatment of cancer is their immunosuppressive effects. Sustained signaling through cAMP is generally immunosuppressive. Specifically, cAMP signaling can lead to the inhibition of NF- κ B, a potent pro-inflammatory mediator, as well as downregulation of TNF- α , IFN- γ , and IL-17 [96]. With the advent of cancer treatment strategies that harness the immune system, such as checkpoint inhibitors and

Fig. 2 Literature screening process. The articles were sorted into three categories: PDE4A, PDE4B, PDE4C, PDE4D, PDE4A/B, PDE4A/C, PDE4A/D, PDE4B/C, PDE4B/D, PDE4C/D, and PDE4A/B/C/D. Articles that cited PDE4 and cancer without referencing specific subtypes and articles that used nonspecific PDE4 inhibitors for pharmacologic knockdown of PDE4 were categorized as “Unspecified”. Articles that examined PDE4 outside the context of cancer were placed in the “Not Relevant” category. For the purposes of constructing a meaningful data analysis, papers from each of the categories with two unique PDE subtypes were added to the individual subtype categories. This led to a final classification of papers as PDE4A ($n = 17$), PDE4B ($n = 34$), PDE4C ($n = 7$), and PDE4D ($n = 44$).



adoptive cell transfer, PDE4 inhibitors should be investigated with caution. It is unclear what effect these inhibitors may have on immunogenic treatments.

Strengths and limitations

Potent, marketed PDE4 inhibitors are available for clinical proof-of-concept studies in cancer as in the case of the published clinical Phase 0/1 biomarker and pharmacodynamic study of roflumilast in patients with advanced B-Cell hematologic malignancies (NCT01888952) [92] and as in an on-going clinical study of roflumilast in combination with standard chemotherapy for high-risk DLBCL (NCT03458546). The underlying therapeutic hypothesis is that overexpression of PDE4B contributes to oncogenesis through downregulation of intracellular cAMP and that this can be opposed by pharmacological inhibition of the cyclic nucleotide phosphodiesterase. The limitation of marketed

PDE4 inhibitors is their poor gastrointestinal tolerability with escalation of dose which may preclude achieving sufficient inhibition of specific PDE4 subtypes for demonstration of clinical benefit [5]. Alternatively, subtype selective PDE4 inhibitors may show better tolerability [6], but to date it has only been possible to target PDE4B [9, 97] or PDE4D [6, 98] with subtype selective inhibitors. A major limitation in structure-based drug design is the absolute amino acid sequence conservation of the PDE4 active site across PDE4 subtypes [99]. Subtype selective PDE4 inhibitors, in contrast, bind in allosteric sites located on regulatory domains that open and close across the PDE4 active site, and these binding sites differ in amino acid sequence between PDE4 subtypes [6, 9, 98]. In addition, specific isoforms protein products translated from the four PDE4 genes have been shown to have unique cellular localizations and functions [100]. It is plausible that these unique proteins may account for some of the discrepancies in PDE4 subtype

functions observed and reported here. Future studies should report PDE4 isoform specificity wherever possible to determine the clinical relevance of PDE4 isoform compartmentalization and local cAMP gradients within cancer cells.

Methods

Search strategy

In order to systematically evaluate the role of PDE4 specific subtypes in cancer, we searched MEDLINE via PubMed for studies that mentioned PDE4 and its association with cancer. Our specific advanced search terms included: “PDE4 and Cancer” OR “PDE4A and Cancer” OR “PDE4B and Cancer” OR “PDE4C and Cancer” OR “PDE4D and Cancer”. Our search results provided 297 papers as of November 1, 2019.

Screening criteria

Two investigators reviewed each initial study to determine whether it was specific for one or more PDE4 subtypes. The articles were sorted into the following 13 categories (Fig. 2): PDE4A ($n = 9$), PDE4B ($n = 26$), PDE4C ($n = 3$), PDE4D ($n = 36$), PDE4A/B ($n = 3$), PDE4A/C ($n = 1$), PDE4A/D ($n = 3$), PDE4B/C ($n = 1$), PDE4B/D ($n = 3$), PDE4C/D ($n = 1$), PDE4A/B/C/D ($n = 1$), unspecified ($n = 93$), and not relevant ($n = 117$). Articles that cited PDE4 and cancer without referencing specific subtypes and articles that used nonspecific PDE4 inhibitors for the pharmacologic knock-down of PDE4 were categorized as “Unspecified”. Articles that examined PDE4 outside the context of cancer were placed in the “Not Relevant” category. Disagreements between reviewers were resolved by re-evaluation and discussion with a third reviewer.

Data consolidation

For the purposes of constructing a meaningful data analysis, papers from each of the categories with two unique PDE subtypes were added to the individual subtype categories. This led to a final classification of papers as PDE4A ($n = 17$), PDE4B ($n = 34$), PDE4C ($n = 7$), and PDE4D ($n = 44$).

Conclusions

The largest current bodies of research with PDE4 subtypes in cancer surround PDE4A, PDE4B, and PDE4D. The general trend seems to be that PDE4 subtypes correspond to

poor prognosis in many tumor types, although there are a small number of studies contradicting this across each of the subtypes. PDE4A and PDE4B have the largest bodies of evidence supporting their utility as targets for hematologic malignancies, where PDE4D may play a larger role in solid tumors. Regardless of the type of malignancy, there is strong evidence supporting PDE4 inhibition as a viable way to potentiate cAMP-mediated inhibition of AKT pathways. PDE4 may be a viable target for anticancer therapies, and this warrants future studies into specific subtypes across malignancies.

Compliance with ethical standards

Conflict of interest MG is an employee of Tetra Discovery Partners, Inc. that has a financial interest in the discovery and development of PDE4B and PDE4D allosteric inhibitors for the treatment of Fragile X Syndrome, Alzheimer’s disease, and other CNS disorders.

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