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ORIGINAL ARTICLE

Inhibition of the adenosinergic pathway: the indispensable part of oncological therapy in the future

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Abstract

In recent years, immunotherapy has produced many unexpected breakthroughs in oncological therapy; however, it still has many deficiencies. For example, the number of patients who are unresponsive to anti-programmed death-ligand 1 (PD-L1), anti-cytotoxic T-like antigen-4 (CTLA4), and anti-programmed death-1 (PD1) therapies cannot be ignored, and the search for an undiscovered immunosuppressive pathway is imminent. Five decades ago, researchers found that activation of the adenosinergic pathway was negatively correlated with prognosis in many cancers. This review describes the entire process of the adenosinergic pathway in the tumor microenvironment and the mechanism of immunosuppression, which promotes tumor metastasis and drug resistance. Additionally, the review explores factors that regulate this pathway, including signaling factors secreted by the tumor microenvironment and certain anti-tumor drugs. Additionally, the combination of adenosinergic pathway inhibitors with chemotherapy, checkpoint blockade therapy, and immune cell-based therapy is summarized. Finally, certain issues regarding treatment via inhibition of this pathway and the use of targeted nanoparticles to reduce adverse reactions in patients are put forward in this review.

Keywords Immunosuppression · Poor prognosis · Adenosine · Targeted nanoparticles

Introduction

To avoid being recognized by the immune system, tumor cells have developed mechanisms such as immune escape and immunosuppressive pathways that protect the tumor and continue to operate from the early stage to the advanced stage [1–3]. According to further research in this field, the immunosuppressive checkpoint molecules CTLA4 and PD1, which are expressed on $CD8^+$ T lymphocytes, are targets to recover the immune response [4]. Currently, ipilimumab and nivolumab can successfully increase the survival of patients with various cancers, and the combination of ipilimumab and nivolumab has shown improved efficacy in patients with non-resectable metastatic melanoma [5, 6]. However, the adverse events caused by immunotherapy and the ineffectiveness of checkpoint inhibitors for certain patients should be seriously considered [7].

In recent years, in the adenosinergic pathway, the ADO (adenosine) generated by the ectonucleotidases CD39 and CD73 has been considered as a new "immune checkpoint mediator" that impairs the function of the immune system [8]. Interestingly, researchers found that regulatory T (Treg) cells are eliminated by checkpoint blockade therapy; however, they release a high amount of adenosine triphosphate (ATP), and CD39 and CD73 quickly transform ATP to ADO that targets T cells to hamper immune checkpoint blockade-mediated immune response [9]. This observation can explain why some patients relapse or experience worsened conditions after checkpoint blockade treatment. In addition, the adenosinergic pathway has an important effect on cancer cells and tumor microenvironment (TME); thus, it is worth considering it as a new target in clinical treatment [10].

Cancer patients have received great benefits from checkpoint blockade therapy, and how to optimize this treatment for more patients and less adverse reactions should be focused on in the next step [6, 7]. It has been shown that inhibitors of the

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adenosinergic pathway have great advantages of solving these difficulties, so we should explore how they can be combined with anti-PD1 and anti-CTLA4 therapies [9]. In this review, we propose to use nanoparticles for improving safety and efficacy of inhibitors of the adenosinergic pathway and also have shown an optimized approach of designing associated nanoparticles.

The adenosinergic pathway in cancer

What role the adenosinergic pathway plays in cancer?

High expression in malignant tumors

In humans, overexpression of CD73 has been shown in various cancers such as ovarian carcinoma, melanoma, breast cancer, colon cancer, and head and neck cancer, and these articles have reported a potential relationship between high CD39/CD73 expression and poor prognosis [11–19], high metastasis [20], and chemoresistance [21–23]. Similarly, this study analyzed publicly available gene expression data that correlated the expression of adenosine A_{2B} receptor (A2BR) with prognosis and found that expression of A2BR was actually correlated with poor prognosis of triple-negative breast cancer (TNBC) patients [24], indicating that A2BR could be considered as a new target in some breast cancers. In addition, another study indicated that high expression levels of the adenosine A_{2A} receptor (A2AR) gene and protein have same prognostic effects in non-small cell lung cancer (NSCLC) [17] (Table 1).

Several aspects of poor prognosis

Drug resistance Drug resistance was involved in poor prognosis; the number of $CD39^+$ Treg cells and level of ADO increased significantly and $CD4^+$ T helper cells decreased after chemoradiotherapy, which could increase the tumor resistance to chemoradiotherapy [33]. Similarly, the EGF inhibitor cetuximab was found to upregulate the level of ADO to increase resistance in HNSCC [34]. Additionally, this study indicated that overexpression of CD73 would confer drug resistance to anthracyclines, such as doxorubicin [22]. Another study found that constant exposure to interferons derived from the adenosinergic pathway can confer an adaptive resistance to checkpoint blockade immunotherapy by inducing PD-L1 expression on tumor cells [35]. Similarly, in HER2/ErbB2 breast cancer, CD73 confers tumor cell resistance to anti-ErbB2 mAb has been reported [36].

Anti-apoptosis Recently, it was observed that CD73 could downregulate the apoptosis of Jurkat leukemia cells mediated

Table 1 The clinical implication of adenosinergic molecules in cancers

Biomarker	Cancer type	Clinical implication	Reference
CD39	Chronic lymphocytic leukemia	High CD39 expression on T cells was associated with poor prognosis	[176]
	High-grade serous ovarian cancer	High CD39 expression was associated with poor OS	[13]
	Gastric cancer	High CD39 expression associated with poor prognosis	[177]
CD73	Gastric cancer	High CD73 expression was associated with poor prognosis	[178]
	Triple negative breast cancer	High CD73 expression was associated with a poor prognosis in TNBC patients but not in luminal or $HER2^+$ breast cancer patients	[22]
	Non-small cell lung cancer	High CD73 expression was associated with poor prognosis	[16]
	Malignant melanoma	High CD73 expression was associated with metastatic site specificity in malignant melanoma	[179]
	Ovarian cancer	High CD73 expression was associated with poor prognosis in patients who have many $CD73^+CD8^+$ T cells in TME (meta-analysis)	[13]
	Rectal adenocarcinoma	High CD73 expression was associated with poor prognosis	[180]
	Colorectal cancer	High CD73 expression was associated with poor prognosis in human CRC	[18, 181]
	Gallbladder cancer	Patients with high NT5E (encoding CD73) expression was associated with poor prognosis	[182]
	Leukemia	High CD73 expression was associated with the development of leukemia subtype	[183]
	Chronic lymphoblastic leukemia	High CD73 expression was associated with poor prognosis	[23]
	Prostate cancer	High CD73 expression was associated with poor prognosis and more metastatic burden	[14, 184]
A2AR	Non-small cell lung cancer	High A2AR gene and protein expression levels have same prognostic effects in non-small cell lung cancer	[17]
A2BR	Triple negative breast cancer	High A2BR expression was significantly associated with poorer survival in TNBC (meta-analysis)	[24]

by tumor necrosis factor-related, apoptosis-inducing ligand (TRAIL) [37]. This effect is not associated with the enzymatic activity of CD73 but associated with the colocalization of CD73 with the TRAIL receptor DR5 [38]. Similarly, this article indicated that CD73 would downregulate the frequency of anti-apoptotic factors which always are BCL2 and BCLxL [39].

Tumor metastasis CD73 has a significant effect on cancer cell growth and invasion [40]. In particular, high CD73 expression has been proposed as a biomarker for high metastasis in melanoma and breast cancer [41–43]. Similarly, this study found that CD73 is both expressed on $MITF^{low}$ and a part of $MITF^{high}$ melanoma cells, and CD73 is considered a significant metastatic biomarker [44]. It was found that myeloid-derived suppressor cells (MDSCs) as source of VEGF which upregulated tumor cell metastasis in melanoma. Some A2BR inhibitors (PSB1115 and PBF-1129) delayed MDSC invasion and downregulated the VEGF concentration has been reported, which strongly reduced angiogenesis in these mice [45].

Immune suppression CD39 has a significant effect on downregulating immune cell response [46]. These studies indicated that TGF-β-cultured Th17 cells expressed CD73 and exhibited the capability to inhibit the immunity function [47, 48]. Similarly, the ADO generated by CD39/CD73 that was expressed on Tregs exhibited strong immunosuppression in these studies [49, 50]. In another study, T cells and MDSCs could produce ADO that activated A2AR, which suppressed the immune response to tumor cells [51].

How does the adenosinergic pathway affect cancer?

Overview of the adenosinergic pathway

In normal physiological tissue, ATP was located in the intracellular compartment at concentrations greater than 1 mM but less than 10 mM and has a low concentration (10–100 nM) in TME [52]. Specially, extracellular ATP concentration rose strongly in response to homeostasis-interfered conditions, including inflammation, hypoxia, tissue stress, chemotherapy, radiotherapy, cell apoptosis, ischemia, and malignancy [11, 53]. Additionally, CD39 can catalyze extracellular ATP into adenosine diphosphate (ADP) and ADP into adenosine monophosphate (AMP). Next, CD73, which is downstream of CD39 in adenosinergic pathway, can convert AMP into ADO (Fig. 1). Importantly, the conversion of ATP into AMP via CD39 is reversible; however, the catalysis of AMP into ADO mediated by CD73 is not reversible [54]. Therefore, CD73 was regarded as the decisive enzyme in the progress of extracellular ADO production [55]. In addition, extracellular ADO can be catalyzed into inosine through ectoadenosine deaminase (ADA) (associated with CD26 in humans) [56] (Fig. 1).

Ectonucleotidases CD39 is widely expressed [57]. Consistent with all nucleoside triphosphate diphosphohydrolases (NTPDases), there are five conserved sequences that are also known as "apyrase conserved regions" on CD73, which participate in active site formation [58] and is important to ectoenzymatic activity [59, 60].

CD73 is a 70-kD, glycosylphosphatidylinositol (GPI) protein located on the cell membrane and is encoded by the NT5E gene [61]. The dissociative form of CD73 is similar to membrane-bound form of CD73 [62, 63]. CD73 has three domains, which contain an N-terminal domain, C-terminal domain, and a short alpha helix [64]. Importantly, functional CD73 must be a homodimer with a non-covalent interaction between adjacent C-terminal domains, which exist in two conformations: open and closed. Switching from the open to the closed conformation is required for ectoenzymatic activity by allowing substrate binding and product releasing [65]. Additionally, the zinc ions are important to the ectoenzymatic activity of CD73 [64, 65]. Additionally, functional glycosylation modifications occurred on CD39, which is also significant for the catalytic activity of CD39 [66]. In this research, it was found that the experiment for depleting membrane cholesterol through drugs exhibited a significant inhibition of the CD39 catalytic activity [57].

Receptors ADO binds to P1 (type 1 purinergic receptors) receptors, a family of G protein-coupled receptors. This family includes four isoforms, A1R, A2AR, A2BR, and A3R [67]. A1R, A2AR, and A3R, which exhibit high activity in a low concentration of ADO (1–100 nM), are high-affinity receptors, but A2BR is a low-affinity receptor that only exhibits activity in surroundings with high levels of ADO, such as TME (25 μM) [39]. Therefore, the host A2BR is unlikely to be involved in metastasis [24]; however, the host A2AR contributes to tumor metastasis and immunosuppression [68]. Activated A2AR and A2BR promote adenylyl cyclase (AC) and the product cyclic AMP to suppress the function of immune cells [69–71]; however, the activated A1R and A3R inhibit cAMP generation to enhance the function of immune cells [72, 73]. A1R has a broad distribution and is particularly abundant in the nerves, heart, and kidneys. A2AR is expressed on cancer, NK cells, $CD4^+$ T cells, B cells, Tregs, CAFs, $CD8^+$ T cells, macrophages, the endothelium, and striatal area. A2BR is distributed in the endothelium, MDSCs, CAFs, DCs, and tumor cells. A3R is expressed in the nervous and cardiovascular systems, immune cell subsets, the liver, and tumor cells. [4].

The mechanism of the adenosinergic pathway

ADO catalyzed by CD39/CD73 acts on a wide range of cells and causes many cascades to contribute to tumor growth, anti-

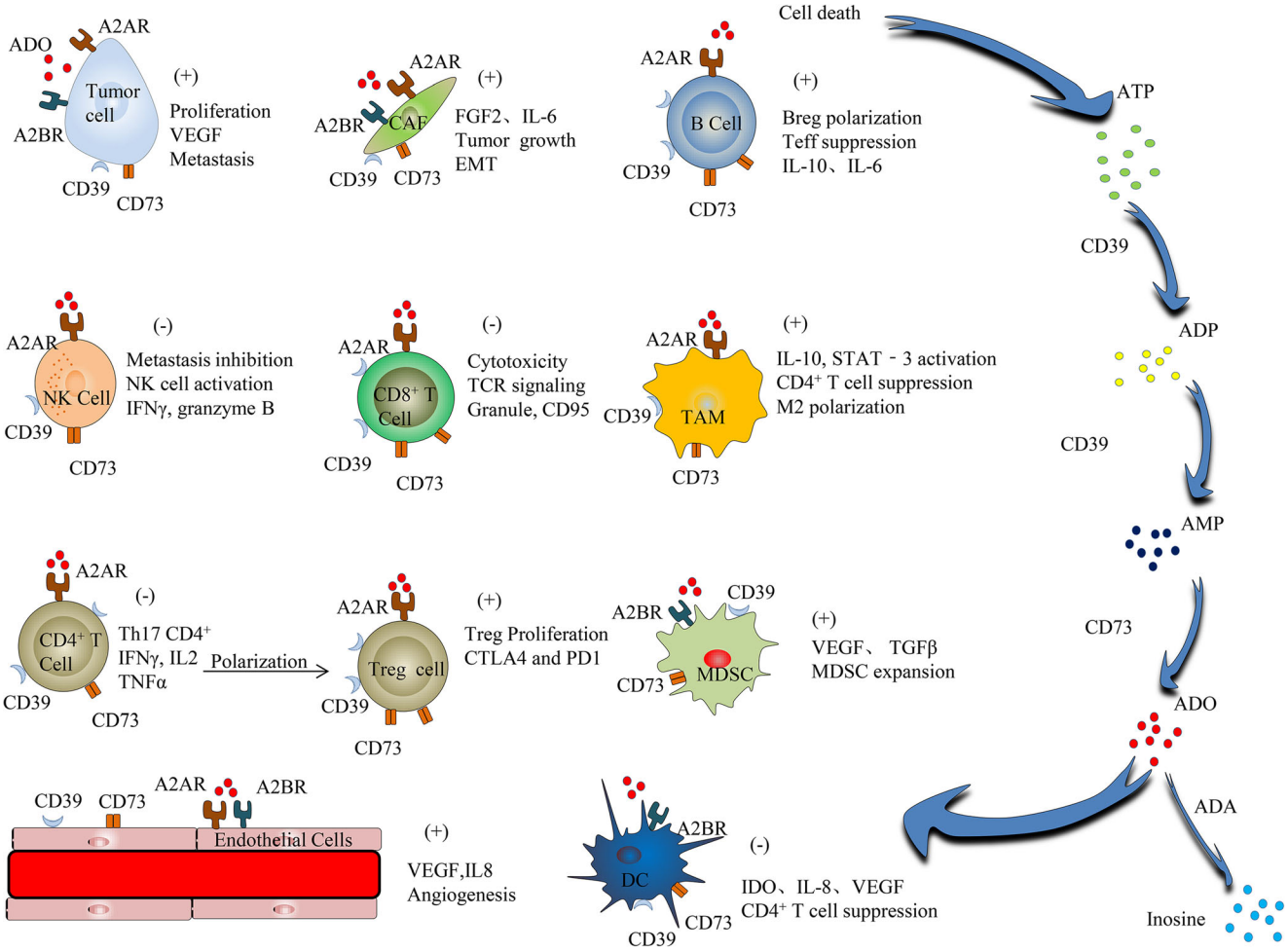

Fig. 1 The entire process of the adenosinergic pathway in the tumor microenvironment and the mechanism of immunosuppression, metastasis and drugs resistance. Note: Positive sign (+) means increasing, Negative sign (-) means decreasing

apoptosis, drug resistance, tumor metastasis, angiogenesis, and immune escape in TME [39].

Tumor cells It has been observed that the inhibition of CD39 can improve development and immunosuppressive function of immune cells in melanoma, ovarian, and TNBC [74–76]. Similarly, the knockdown of A2BR in tumor cells decreased VEGF production and lung metastases and even reduced tumor cell proliferation and invasion [24]. Additionally, it activated A2BR in TNBC cells and enhanced their invasion and proliferation through the ERK signaling pathway. Another study indicated that A2BR as an activated form is important to growth and survival of tumor [71]. Interestingly, both host- and tumor-expressed CD73 are critical to immunosuppression. And knockdown or overexpression of CD73 on tumor cells can affect the proliferation and metastasis in cancer [75, 77]. Additionally, another study indicated that the overexpression of A2BR on tumor cells promotes metastasis [24].

CAFs Activated A2BR on cancer-associated fibroblasts (CAFs) can upregulate the frequency of fibroblast activation protein (FAP)-positive fibroblasts. Additionally, activated A2BR results in the enhanced production of CXCL12, which is released by FAP-positive fibroblasts, and CXCL12 acts on tumor cells to release VEGF, which can increase the number of $CD31^+$ endothelial cells [78]. Activated A2BR on CAFs can promote the protein kinase C (PKC) signaling to release interleukin-6 (IL-6), which is important for the epithelial-to-mesenchymal transition (EMT) [39]. In addition, this study has shown that fibroblasts can promote Tregs via ectoenzymatic activity of CD73. α,ß-Methylene adenosine-5′-diphosphate (APCP), a specific CD73 inhibitor, and an anti-CD73 mAb (AD2) can inhibit CD73 and are applied to promote $CD8^+$ T cells [79, 80]; additionally, this study indicated that therapy via inhibiting CD73 can restore the function of immune cells.

$CD4^+$ T cells/Treg cells Several studies have shown that CD39 expressed on $CD4^+$ T cells can downregulate IL-17 production by suppressing the function of T cells [81, 82] and can inhibit that T cells become Th17 cells [83, 84]. Additionally, in these studies, enhanced levels of $CD39^+$ Treg cells, which

impaired T cell immunity, were recognized in AT3 mammary carcinoma model [85, 86]. In another study, $CD39^+CD8^+$ T cells always exhibited the enhanced frequency of checkpoint molecules such as PD1 and CTLA4 [87]. Interestingly, it has been shown that $CD4^+FOXP3^-$ T cells exhibited a high CD73 expression in mouse model [88]. The frequency balance between Treg cells and T helper cells could be mediated by adenosinergic pathway inhibitors in mice with malignant tumor [89]. In the TME, it had been shown that increased number of $CD39^+CD4^+$ T cells and the presence of $CD73^+CD4^+$ T cells expressing immune checkpoints were strongly associated with immunosuppression in breast and ovarian tumors [90].

B cells Both CD39 and CD73 and adenosine receptors are co-expressed on human B cells. The $CD39^+CD73^+$ B cell subtype is associated with decreased immunity via $CD4^+/CD8^+$ effector T cells [91]. It has been observed that human regulatory B cells (Bregs) with high CD39 expression have a high proliferative capacity and produce a large amount of ADO and IL-10 [92]. It was found that ADO and agonists of B cell receptors (BCR) and toll-like receptors (TLR) can co-stimulate B cells and elevate the frequency of these cells to convert B cells to class-switched B cells. Additionally, high CD73 level was correlated with enhanced Breg [93]. In addition, it was illuminated that the ADO-producing B cells secreted IL-6, which contributed to tumor cell proliferation [9] and that the adenosinergic pathway was involved in inhibition of T cells [40].

$CD8^+$ T cells Recently, A2AR activated by ADO that regulated the intracellular Wnt-signaling has been reported, which led to the blockade of the conversion of immature $CD8^+$ T cells into activated T cells [94, 95]. Another study found that CD73 expression influenced the reliability of the prognosis assessment of infiltrating $CD8^+$ T cells in highgrade ovarian cancer in the clinic [13]. In addition, genetic ablation of A2AR and inhibition of A2AR via antagonists could enhance immune cell-based therapy that restored immunity in the TME [70]. Similarly, it has been indicated that A2AR located on tumor cells intrinsically regulated the frequency of $CD8^+$ T cells in the TME [77, 96, 97].

NK cells It has been indicated that the expression of CD73 on human NK cells increased after these cells contacted mesenchymal stromal cells (MSC) and that this could be involved in a follow-up immunosuppressive process [98]. On the other hand, only A2AR, and no A2BR, was expressed on NK cells, and its frequency was greatly enhanced under certain pathological stimuli such as sickle cell disease (SCD) [99]. Subsequently, upregulated A2AR contributed to the inhibition of activated NK cells and their cytotoxicity function [87]. Moreover, we have tested this idea by detecting the expression of granzyme B on NK cells isolated from A2AR-antagonist-treated mice, and it was found that the frequency of granzyme B was enhanced. Interestingly, it was indicated that the upregulation of A3R was correlated with enhanced NK cell activity [100]. In summary, A2AR signaling suppressed natural killer cell maturation and contributed to the metastasis of $CD73^+$ tumors in the tumor microenvironment [101].

TAMs It has been found that CD39 on monocytes could inhibit the chemotaxis, adhesion, and trans-migration capacity of these cells [102]. Compared with immunosuppressive M2 macrophages, M1 macrophages expressed lower frequency of CD39 and CD73 [103]. In associated studies, it was indicated that the CD73 inhibitor APCP increased the proportion of M1 macrophages between these macrophages [104]. Similarly, downregulated M2 macrophages have also been found in CD73-deficient mice [105]. In contrast, ADO binding to A2AR and A2BR contributed to impairment of macrophage function. For example, the increased level of M2 markers and IL-10-induced STAT-3 via the adenosinergic pathway has been shown to suppress $CD4^+$ T cell proliferation [106–108].

MDSCs The inhibition of A2BR could prevent the migration of $CD11b^+Gr1^+$ myeloid-derived suppressor cells (MDSCs) and could downregulate VEGF levels, which are important for reducing angiogenesis [45]. In contrast, it was found that agonists of A2BR contributed to infiltration and expansion of MDSCs, and the potential mechanism could be phosphorylation of STAT-3 [109, 110].

Which factors regulate the adenosinergic pathway

Endogenous factors Certain endogenous molecules have an important impact on the adenosinergic pathway. It was found that IL-27 contributed to the expression of CD39 on DCs via STAT3 and aryl hydrocarbon receptor (AhR) [46]. TNBC, ovarian cancer, and colorectal cancer often carried the TP53 mutation [111]. And TP53 mutation was positively associated with the epithelial-to-mesenchymal transition (EMT). Consequently, tumor cells underwent EMT, which exhibited enhanced expression of CD73 on the cell surface [36]. In addition, the expression of A2AR and A2BR was increased by tumor necrosis factor (TNF), due to activated NFκB pathway [112, 113]. Interestingly, HIF-2α but not HIF-1α in human lung endothelial cells promoted the expression of A2AR, and siRNA knockdown of HIF-2α completely downregulated the frequency of A2AR [114]. On the other hand, TLR4 (toll-like receptor 4)-dependent inflammation caused a significant improvement of A2AR and A2BR levels in tumor-associated macrophages (TAMs) [115]. Moreover, it was indicated that transforming growth factorβ (TGFβ) and hypoxia-inducible factor (HIF)-1α could profoundly upregulate the level of

A2AR, CD39, and CD73, which is located on both $CD8^+$ and $CD4^+$ T cells [39].

Exogenous factors In addition to endogenous molecules, exogenous treatments also took part in the progress of immunosuppression, metastasis, and drug resistance caused by the adenosinergic pathway. Metformin inhibits MDSC activity in patients with ovarian cancer by decreasing the levels of CD39 and CD73 [116]. In contrast, CD40 mAb, as an agonist, enhances CD73 and CD39 expression; however, the mechanism is not very clear [86]. Additionally, it has been shown that CD73 expression on tumor cells in a subset of patients with melanoma increases after receiving antiPD1 immunotherapy and that it impairs the treatment effect [44]. Similarly, another study has reported that carboplatin, doxorubicin, gemcitabine, and paclitaxel induce enrichment of $CD47^+/CD73^+/PDL1^+$-immune-evasive tumor cells in TNBC [117]. Triiodothyronine (T3) treatment causes a significant enhancement of CD73 expression in a dose–response manner in C6 rat glioma cells and smooth muscle cells, and this effect may be caused by thyroid follicular cell hyperfunction [118, 119]. Interestingly, in rats receiving chronic stimulation via a low-iodine diet or treated with propylthiouracil, the enzymatic activity of CD73 is augmented [120]. Moreover, LEC (low-dose endotoxin conditioning) treatment of RPMΦ (resident peritoneal macrophages) causes an ~3-fold increase in the A2AR level and an ~28-fold increase in the ADO level [121].

Current status of treatment of adenosinergic pathway

Combination therapy

Combination with checkpoint blockade treatment Currently, a research indicated that antiPD1 therapy upregulated A2AR expression on $CD8^+$ T cells in cancer. Similarly, it was indicated that CD73 expression increased in patients with acquired resistance to antiPD1 therapy, which showed that the adenosinergic pathway was involved in the impairment of immune checkpoint blockade therapies [47]. It has been shown that the simultaneous blockade of PD1 and A2AR upregulated the frequency of $CD8^+$ T cells and NK cells, which could inhibit the metastasis of tumor cells [122]. In addition, the combination of CD73 mAb (MEDI9447) and antiPD1 therapy impaired the growth of mouse CT26 colon carcinoma [123]. Additionally, it has been shown that the CD73specific inhibitor APCP and antiCTLA4 profoundly reduced melanoma survival compared with monotherapy [124]. In another study, B7DC-Fc fusion protein could reduce checkpoint molecule-mediated immunosuppression, which improved the survival of mice [125]. It also indicated that combination therapy conferred a prolonged survival to mice [125].

Combination with chemotherapies It has been reported that the blockade of CD73, A2AR, or A2BR enhances the effect of chemotherapy [22, 24, 126]. This indicates that inhibition of the adenosinergic pathway contributes to the enhancement of chemotherapy [22]. It has been shown that high CD73 level in patients is correlated with upregulated resistance to anthracycline [22]. It has also been elucidated that combination therapy with doxorubicin and anti-CD73 or A2AR antagonists contributes to prolonged survival in mice. Similar results have demonstrated that the blockade of CD39 on fibrosarcomas restores sensitivity to anthracyclines [127]. Moreover, a higher efficacy has been reported with the combination of A2BR inhibitors with chemotherapeutic drugs such as dacarbazine and doxorubicin, which showed similar results to those described above [24, 126].

Combination with immune cell-based therapy In addition to conventional chemotherapies and checkpoint blockade treatment, the combination of the inhibition of the adenosinergic pathway and $CD8^+$ T cell-based therapies is a significant strategy [128]. Antigenspecific T cells in combination with APCP enhanced the impairment of tumor growth compared with monotherapy [128]. It has been shown that the inhibition of A2AR significantly improved the treatment effect of chimeric antigen receptor (CAR) T cells on mammary tumor cells [129], which was explained by enhanced levels of CAR T cells and cytokines released by T cells [129]. In addition to T cell-based therapies, a high A2AR frequency has been reported on NK cells, and the suppression of A2AR could significantly enhance NK cells in malignant cancers [77, 122]. Thus, NK cell-based therapies were enhanced by blocking the activation of A2AR [39].

Drugs that have entered clinical studies

In recent years, many small molecule drugs or antibodies inhibiting the adenosinergic pathway are undergoing clinical trials. Their targets include CD73, A2AR, and A2BR but not CD39, and most of these inhibitors are applied to malignant tumors such as ovarian cancer, NSCLC, and other advanced cancers. Additionally, they will also be used in combination with checkpoint blockers such as anti-PDL1 and anti-PD1 to achieve better efficacy (Table 2). Many researchers and medical companies wait for the first clinical trial results, which would come from patients who are receiving inhibitors that reduce extracellular ADO generation or activity in tumors. These observations present a good opportunity to develop anti-CD73 therapy for the treatment of certain cancer patients. Based on this knowledge, although there is still a long way to go, future studies can be expected that aim at translating anti-CD73 therapy for cancer patients in the clinic.

Table 2 Clinical trials of antagonists of the adenosinergic pathway

Target	Drug	Clinical trial number	Cancer type	Combination partner	Study phase	References
CD73	MEDI9447	NCT02503774	Solid tumors	Anti-PDL1 (MEDI4736)	Phase 1	[25]
		NCT03267589	Ovarian cancer	Anti-PDL1 (durvalumab)	Phase 2	[26]
	CPI-006	NCT03454451	Advanced cancers	Anti-PDL1 (pembrolizumab) A2ARi(CPI-444)	Phase 1/1b	[27]
A2AR	NIR178	NCT03207867	Solid tumors and non-Hodgkin lymphoma	Anti-PD1 (PDR001)	Phase 2	[28]
	PBF-509	NCT02403193	NSCLC	Anti-PD1 (PDR001)	Phase 1, phase 2	[29]
	CPI-444	NCT02655822	Advanced cancers	Anti-PDL1 (durvalumab)	Phase 1/1b	[30]
	AZD4635	NCT02740985	Advanced cancers	Anti-PDL1 (durvalumab)	Phase 1	[31]
A2BR	PBF-1129	NCT03274479	NSCLC	N/A	Phase 1	[32]

What should we do for a better therapeutic effect in the future?

Because A2AR/A2BR and CD39/CD73 are important to maintain the health and homeostasis in normal tissues, the ubiquitous effects of drugs in the body will destroy these intact systems and cause unnecessary or even fatal toxic side effects. For example, a study has shown that some cells have a high expression of efflux pump P-glycoprotein (P-gp), which pumps out drugs to promote drug resistance; activating A2AR via an FDA-approved A2AR agonist (Lexiscan) significantly decreased P-gp expression and function to enhance the drug efficacy in a time-dependent manner. This suggests that medication without delivery systems targeting tumor sites may cause some serious adverse reactions [130]. Similarly, the ubiquitous expression of CD73 has a significant effect in adjusting the dynamic balance between ATP and ADO to maintain physiological homeostasis; therefore, drug delivery without tumor-targeting may decrease effective metabolic activity of CD73 [131]. Another article has reviewed that CD73-KO mice exhibits functional defects in multiple aspects [132]; for example, intestinal epithelium motility and permeability, reducing damage associated with ischemia, hypoxia-induced vascular leakage, renal function, leukocyte trafficking, immunity and endothelial barrier function, are downregulated compared with wild-type mice [133–143]. Therefore, care should be taken during treatment, considering that an antagonist of CD73 could cause potential damage to patients who receive adenosinergic pathway blockade treatment in the clinic [132]. On the other hand, formulations with no carrier are unstable for drug delivery to tumor sites. Many cells, such as macrophages, can clear the drug, and other molecules can neutralize the active ingredients via processes such as opsonization. In addition, drugs without nanoencapsulation will be rapidly metabolized by the liver and excreted by the kidneys, which will result in a very low half-life and will greatly reduce the efficacy of the drug [144]. Overall, to enhance the efficacy and reduce the side effects of adenosinergic pathway blockade drugs, the use of tissue- and cell-targeting systems has become the focus of future drug development in this area.

The advantages of nanoparticles for delivering inhibitors of the adenosinergic pathway

In short, the original intention of developing drug delivery systems was to improve the therapeutic effect by altering the transport process for drug molecules in the body. The optimization of drug delivery systems focused on aspects including formulation, controlled release, delivery routes, and half-life, which could significantly enhance treatment efficacy [145–147]. In recent years, drug delivery systems have been developed quickly and efficiently, and nanoparticles (NPs) gradually have become the most popular drug-carrier [147]. Scientists highly recommend nanoparticles because of multiple aspects. There are many efficient and convenient manufacturing methods (solvent displacement, salting out, emulsion diffusion, emulsion–solvent evaporation, in situ polymerization) to form a wide variety of NPs such as micelles, liposomes, magnetic nanoparticles, gold nanoshells, carbon nanotubes (CNTs), nanohydrogels, and dendrimers, which can effectively cope with various diseases [148–150]. They possess the capacity to carry multiple types of insoluble drugs [149]. For example, NP formulations of hydrophobic drugs such as paclitaxel can overcome the difficulties of drug insolubility in water [151]. Efficient delivery systems could be applied in diagnostics in a multitude of diseases [152]. NPs exhibit increased drug-loading capacity due to their large surface area to volume ratio [153]. NPs exhibit a longer systemic circulation than conventional systems and reduce clearance by the kidneys. NPs can protect drug molecules from attack by the host immune system and enzymatic degradation, and certain immune-modifications on NPs can inhibit the suppressive TME, which is associated with tumors, for enhancing cancer chemotherapy and reducing drug resistance. NPs extravasate into tissues from leaky blood vessels more easily compared with drug molecules. Combining this factor with the enhanced permeation and retention (EPR) effect reduces the challenges

of off-target effects caused by increasing the drug concentration [154, 155]. In addition, NPs, such as liposomes, can avoid drug efflux pumps to weaken multidrug resistance (MDR), which will enhance the anti-tumor drug accumulation in tumor cells. Additionally, safer and less expensive nanocarriers are constantly being developed and innovated to achieve more effective drug delivery and improved efficacy [156]. Targeted delivery systems have been studied for delivering adenosine pathway antagonists [157], and it has been found that CD73-specific siRNA-loaded chitosan-lactate nanoparticles (ChLa NPs) can lead to reduced expression of CD73 in tumor cells. This decreases tumor growth and metastasis and improves mice survival, thus enhancing the efficacy of DC-based cancer immunotherapy compared with naked CD73-specific siRNA and eventually achieving the downregulation of Tregs, MDSCs, and TAMs in the TME. Therefore, the potential of NPs has been identified to improve the efficiency of drug delivery and to decrease the side effects associated with present therapeutics.

Applying nanoparticles in combination therapy

In the earlier part of this review, the combination of adenosinergic pathway blockade and other therapies (checkpoint blockade treatment, chemotherapy and immune cell-based therapy) has been elucidated in detail, and combination therapy is expected to show great prospects. However, it is worth exploring a strategy to achieve an effective cooperation of two or even more drugs. The emergence of NPs has solved this problem because NPs can effectively combine the effects of multiple drugs and serve as a multilayered, synchronous, and collaborative drug delivery system. In addition, theoretically, the NPs can be used in evaluating the efficacy of combinatorial therapy via designing multiple drug-loaded particles. Compared with separated drug administration, co-delivery of two or more drugs in a carrier has some significant merits, including (1) reduce drug dosage, (2) significantly improves patient compliance, and (3) controls the individual dosage [158]. In this study, the application of nanoparticle for combination therapy confers several potential advantages such as good biodistribution, enhanced blood stability, controllable drug release, high carrier capacity, prolonged systemic circulation lifetime, and multidrug encapsulation for combination therapy [159]. Some researches indicated that co-delivery of two therapeutic agents has a synergistic effect, and it could enhance the efficiency of delivering two drugs to the same cell population by at least one order of magnitude when compared with delivering them in two separate carriers. It is important to recognize the relationship between the mechanisms of each agent, pharmacological activity, and the mode of delivery. Conversely, the propound understanding could contribute to the better design of co-delivery vectors with proper timing and sequence of delivery of individual drugs of the combination [160].

The design of active targeted nanoparticles

Compared with passive targeting, active targeting (also known as ligand-mediated targeting) involves modification of the surface of NPs with affinity ligands for specific retention and uptake by the targeted cells. Therefore, it is urgent to seek appropriate modifiers to be applied in NPs for more effective adenosinergic pathway blockade therapy. Representative ligands include antibodies, proteins, peptides, nucleic acids, sugars, and small molecules such as vitamins [161]. Thus, using antagonists of CD39, CD73, A2AR, and A2BR as modifiers on NPs could be a good choice according to the targeted therapy theory [161]. At present, multiple inorganic analogues have been shown to effectively bind to CD73 and potently inhibit CD73 enzymatic activity, including non-hydrolyzable AMP analogs such as APCP, flavonoid-based compounds such as quercetin, and purine nucleotide analogs such as tenofovir and sulfonic acid compounds [162–164]. In addition, several proteins have been identified such as concanavalin A and tenascin C, which can potentially inhibit CD73 enzymatic activity [165, 166]. It is worth studying intrinsic protein inhibitors, including various monoclonal antibodies targeting CD73 and TY/23, which specifically targets murine CD73 [167–169]; other monoclonal antibodies targeting human CD73 include IE9, 7G2, 4G4, and AD2 [63, 80, 167, 168]. Similarly, antagonists of CD39 such as POM1 [8], inhibitors of A2AR such as ZM241385, and antagonists of A2BR such as PSB1115 should also be considered as modifiers on NPs for active targeting [39]. The targeted therapy theory put forward by Paul Ehrlich in 1906 conceived of a selective delivery of effective drugs. For example, chemotherapy is ubiquitously toxic to tumor and normal tissues. There would be excellence in that these toxic agents are only delivered to the cancer tissues and cancer-related tissues to kill cancer cells with minimal adverse reactions. Therefore, it is significant that active targeting drug delivery enables the targeted delivery of agents to specified tissues via active ligands [170].

Conclusion and prospect

This review has addressed the whole process of the adenosinergic pathway in the TME. The pathway-associated enzymes (CD39 and CD73) and activated receptors (A2AR and A2BR) play roles in promoting tumor growth, metastasis, immunosuppression, and drug resistance through multiple mechanisms. Endogenous factors (IL-27, TGFβ, HIF-1/2α, TP53 mutation, EMT transcription factor, TNF, and TLR4) and exogenous factors

(metformin, CD40 mAb, antiPD1, carboplatin, doxorubicin, gemcitabine, paclitaxel, triiodothyronine, low-iodine, propylthiouracil and low-dose endotoxin), which can regulate adenosinergic pathway-associated molecules, have also been summarized. Next, the three major treatments that inhibit this pathway in combination with other therapies such as checkpoint blockade, chemotherapy, and immune cell-based therapy will greatly improve the efficiency of treatment, and this approach will be the focus of future treatments. Most importantly, because many enzymes and receptors in the pathway are widely distributed throughout the body, using the drug by itself may result in many unavoidable side effects. Therefore, the best choice is to encapsulate these antagonists via nanocarrier systems to reduce unnecessary drug distribution. Passive and active targeted nanocarrier systems is significant in cancer therapy, and finding suitable targeting modifications according to the targeted therapy theory could prove to be highly efficient. This review additionally clarifies the direction of future treatments in this area.

To apply the inhibitors of the adenosinergic pathway via a more effective and safe approach in the future, certain problems should be focused on and solved. It is important to investigate whether the suppression of A2AR can enhance the susceptibility of A2BR to ADO, and conversely, whether the suppression of A2BR can enhance the susceptibility of A2AR to ADO. The low A2AR level would downregulate A2BR expression in splenocytes [171], which needs further study to clearly explain the intrinsic mechanism. It remains unknown whether the therapeutic effect of CD73 inhibition is similar to that of A2A/A2B inhibition. It may be possible that blocking CD73 will be more efficient than blocking A2A/A2B receptors due to the combined inhibition of enzymatic and non-enzymatic functions of CD73. It may be more effective to induce activation of the CD26 pathway plus inhibition of the CD73 pathway for adenosinergic pathway blockade than inhibition of the CD73 pathway alone. (CD26-associated ADA can degrade ADO, while CD73 generates ADO.) When selecting patients for clinical trials or selecting experimental tumor models for animal experiments, we should know how to select appropriate subjects for a better experimental outcome. High expression levels of relevant biomarkers, including CD39, CD73, A2AR, and A2BR, should be the standard for better choice. NECA, 5′-(*N*-ethylcarboxamido) adenosine, a non-specific ADO receptor agonist, was identified to upregulate caspase-3 and cause tumor cells apoptosis in cancers which have a high A2BR expression [172]. Additionally, the NECA was also identified to upregulate caspase-dependent apoptosis caused by chemotherapeutics in osteosarcoma [173]. Reversely, the activation of A2BR was identified to improve growth of prostate cancer [174] and glioblastoma [175]. We should clearly define the different mechanisms that are involved. Overall, we believe that the inhibition of the adenosinergic pathway will result in a good outcome in clinical applications and will become an indispensable part of oncological therapy in the future.

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Compliance with ethical standards

Conflict of interest Yi Huang declares that he/she has no competing interest.
Zili Gu declares that he/she has no competing interest.
Yang Fan declares that he/she has no competing interest.
Guangxi Zhai declares that he/she has no competing interest.
Xiaogang Zhao declares that he/she has no competing interest.
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