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Regulation of CD73 on NAD metabolism: Unravelling the interplay between tumour immunity and tumour metabolism

Jianhao Zhan[1,2†], Le Huang[1,2†], Liyan Niu[2], Wenhui Lu[1], Chengpeng Sun[2], Shanshan Liu[3], Zijun Ding[4] and Enliang Li[1*]

Abstract

CD73, a cell surface-bound nucleotidase, serves as a crucial metabolic and immune checkpoint. Several studies have shown that CD73 is widely expressed on immune cells and plays a critical role in immune escape, cell adhesion and migration as a costimulatory molecule for T cells and a factor in adenosine production. However, recent studies have revealed that the protumour effects of CD73 are not limited to merely inhibiting the antitumour immune response. Nicotinamide adenine dinucleotide (NAD+) is a vital bioactive molecule in organisms that plays essential regulatory roles in diverse biological processes within tumours. Accumulating evidence has demonstrated that CD73 is involved in the transport and metabolism of NAD, thereby regulating tumour biological processes to promote growth and proliferation. This review provides a holistic view of CD73-regulated NAD + metabolism as a complex network and further highlights the emerging roles of CD73 as a novel target for cancer therapies.

Keywords CD73, NAD metabolism, DNA damage repair, Target therapy

Introduction

CD73, also known as ecto-5'-nucleotidase, catalyses the hydrolysis of adenosine monophosphate (AMP) to adenosine (ADO). ADO is an important signalling molecule in the tumour microenvironment. In contrast to ATP, which has a proinflammatory effect, ADO has a strong immunosuppressive effect after binding to specific receptors [1]. ADO activates cAMP signalling by binding to the high-affinity receptor A2AR and low-affinity receptor A2BR (both of which are G protein-coupled receptors, GPCRs) on immune cells, leading to the suppression of immune cell functions and infiltration [2]. In most tumour cells, CD73 is abnormally upregulated, converting ATP (which has proinflammatory effects) into ADO (which has immunosuppressive effects), thus inducing immunosuppressive effects in the tumour microenvironment (TME) and mediating tumour immune escape [3].

However, recent studies have shown that the protumour effect of CD73 is not limited to mediating immune suppression through adenosine. Knocking out CD73 in immunodeficient mice can still significantly inhibit tumour growth and proliferation [4, 5]. In addition, the CD73/adenosine pathway is not the only way for CD73 to promote tumour progression. In tumour cells with

† Jianhao Zhan and Le Huang contributed equally to this work.

*Correspondence:
Enliang Li
lienliangyx@163.com
[1]Department of General Surgery, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, 1 Minde Road, Nanchang 330006, China
[2]HuanKui Academy, Nanchang University, Nanchang 330006, Jiangxi Province, China
[3]School of Basic Medical Sciences, Nanchang University, Nanchang 330006, Jiangxi province, China
[4]School of Ophthalmology and Optometry, Nanchang University, Nanchang 330006, Jiangxi, China

adenosine receptor inhibition, high expression of CD73 can still promote tumor migration [6] and improve tumor metabolic adaptability [7]. CD73 can also promote tumor growth, migration, and invasion through pathways other than the adenosine pathway, such as EGFR [8, 9]、MAPK [10], PI3K/AKT [11] signaling pathways. Therefore, activating the adenosine pathway to inhibit tumour immunity is not the only method by which CD73 promotes tumour progression. Exploring the role of CD73 in other aspects of tumours is of great clinical significance for CD73-targeted cancer therapy.

In terms of the function of CD73 itself, CD73 is a metabolic enzyme that regulates the metabolism of various substances within tumour cells. Therefore, one could hypothesize that another pathway through which CD73 exerts its protumour effect is by altering the metabolism of certain specific substances in tumour cells. In recent years, some studies have shown that CD73 is involved in the transport and metabolism of nicotinamide adenine dinucleotide (NAD) [7, 12–15]. NAD is a cofactor involved in multiple redox reactions, such as electron transfer in mitochondria, and plays a vital role in substance metabolism, cell death, DNA repair, and gene expression [16]. Studies have shown that NAD is upregulated in a variety of human malignant tumours and participates in metabolic processes in tumour cells, mitochondrial energy regulation, DNA repair, and other biological processes [17]. In addition, as an important cofactor, NAD affects the activity of multiple key enzymes, thereby playing important regulatory roles in tumour glycolysis, oxidative phosphorylation, and aspartate synthesis [18].

In this review, we review the evidences that CD73 promotes tumour growth and proliferation by increasing the intracellular NAD concentration to regulate the metabolic processes of tumours [7]. Therefore, in addition to mediating immunosuppression, the adaptability of CD73 to tumour metabolism is also crucial for tumour growth. We fully discuss the role of CD73 in tumour NAD metabolism and explore the mechanism underlying the tumour-promoting effect of CD73 from another perspective.

Hypoxia in the tumour microenvironment promotes the expression of CD73

Gene expression data from cancer patient cohorts revealed significant upregulation of CD73 mRNA in various types of cancer compared to normal cells. High CD73 expression indicates a significantly increased risk of developing cancer, and the expression level of CD73 is closely associated with prognosis [19]. Therefore, as a protumour factor, high expression of CD73 promotes tumour initiation and progression. It is unclear why tumour cells have upregulated CD73 expression compared to normal cells. Studies have shown that hypoxic conditions in the tumour microenvironment may be one of the reasons for the high expression of CD73 in tumour cells. The regulatory effect of hypoxia on CD73 depends on the activity of hypoxia inducible factor 1-alpha (HIF-1α) [20]. HIF-1α is an important transcription factor in the hypoxic tumour microenvironment. Under normoxia, HIF-1α is oxidized by intracellular oxygen, leading to its degradation by the proteasome. Therefore, HIF-1α in tumour cells is stabilized only under hypoxic conditions [21]. Research has shown that HIF-1α is a transcriptional regulator of CD73. The stabilization of HIF-1α under hypoxia can directly promote CD73 expression [20]. In addition, hypoxia can regulate the expression of CD73 by altering the metabolic state of tumours. Whether pyruvate is oxidatively phosphorylated by entering the mitochondria or metabolized to lactic acid by lactate dehydrogenase (LDH) in the cytoplasm largely depends on the hypoxic state of the cell. Under hypoxia, tumour cells are more inclined to produce energy via glycolysis to adapt to a lack of oxygen [22]. Lactate is one of the main metabolic byproducts of glycolysis. Recent studies have revealed that lactate can modify the CD73 promoter through histone lactylation (enrichment of histone H3 lysine K18 lactylation (H3K18la) at the CD73 promoter site), and this lactylation modification directly promotes CD73 transcription and reduces the antitumour effect of CAR-T cells [23]. Therefore, the accumulation of lactate under hypoxia also indirectly promotes CD73 expression. It has been demonstrated in vitro that hypoxia and acidity induce elevated mRNA and protein levels of CD73 in tumour cells. High CD73 expression in tumour cells is directly correlated with LDH and HIF-1α activity [24].

Hypoxia-induced upregulation of CD73 has implications for tumour growth. CD73 is regarded as both a signalling cofactor and a therapeutic target involved in hypoxic tissue injury. Hypoxia- and inflammation-induced tissue injury typically releases large amounts of ATP into the extracellular compartment as a "danger signal" to mediate immune stimulation [25]. This immune activation is clearly detrimental to tumour growth. Therefore, tumour cells in a hypoxic environment avoid excessive inflammatory damage and mediate immunosuppression in the tumour microenvironment by upregulating CD73, which converts proinflammatory ATP released by hypoxic tissue injury into adenosine with inflammatory inhibitory effects [26]. Indeed, tumours use this protective mechanism to evade the immune response. Most hypoxic tumours exhibit increased extracellular adenosine concentrations, even up to the millimolar range [27] (Fig. 1).

Hypoxia can maintain HIF-1α activity while allowing cells to favour aerobic glycolysis over oxidative

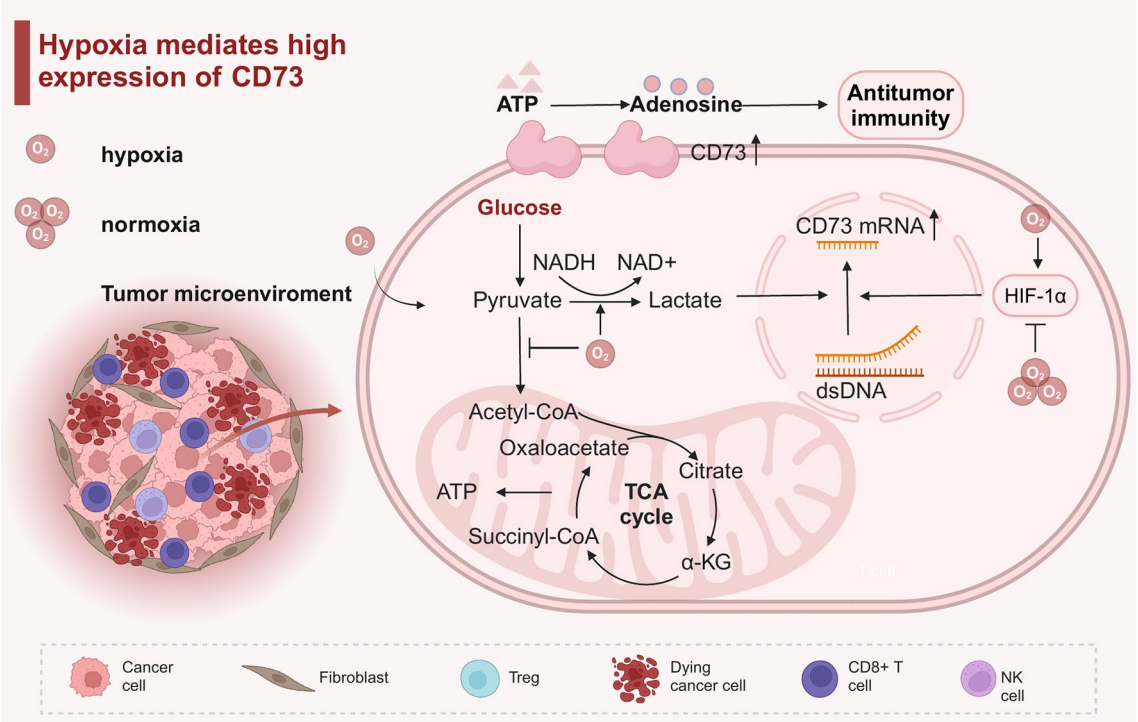

Fig. 1 Hypoxia in the tumour microenvironment promotes the expression of CD73

phosphorylation for energy acquisition. HIF-α can directly promote the transcription of CD73. In addition, aerobic glycolysis leads to the accumulation of large amounts of lactate, which contributes to the formation of lactate in the promoter region and promotes the expression of CD73.

CD73 regulates tumour NAD metabolism

In the previous discussion, we explained how hypoxic conditions in the tumour microenvironment promote the expression of CD73 to facilitate tumour growth. Now, we will elucidate the mechanism by which high CD73 expression exerts its tumour-promoting effects from the perspective of CD73-mediated NAD metabolism regulation.

The process of intracellular NAD synthesis

In mammals, NAD synthesis occurs mainly through three pathways: the de novo pathway, salvage pathway, and Preiss-Handler pathway. The precursors for these three processes, including nicotinic acid (NA), nicotinamide (NAM), nicotinamide riboside (NR), and tryptophan (Trp), are mainly obtained through the diet. Trp is the raw material for the de novo synthesis pathway. Trp is first converted to quinolinic acid (QA) through several steps and then to nicotinamide mononucleotide (NAMN) under the catalysis of quinolinic acid phosphoribosyltransferase (QPRT) [28]. In the Preiss-Handler pathway, NA is converted to NAMN under the catalysis of nicotinic acid phosphoribosyltransferase (NAPRT) [29]. NAMN, the common product of the above two pathways, is converted to nicotinic acid adenine dinucleotide (NAAD) under the catalysis of nicotinamide phosphoribosyltransferase (NAMPT) and is finally converted to NAD under the catalysis of cytoplasmic NAD synthase [30]. The salvage pathway is the main source of NAD. First, NAM is converted to NMN under the catalysis of nicotinamide phosphoribosyltransferase (NAMPT) [31]. NMN is subsequently converted to NAD via the catalysis of ATP-dependent nicotinamide mononucleotide adenylyltransferase (NMNAT). Since NAM is a degradation product of NAD, this pathway involves the recycling of NAD [32].

CD73 promotes intracellular NAD synthesis

Recent studies have confirmed that CD73 promotes the accumulation of intracellular NAD [7, 13]. However, as an extracellular 5'-nucleotidase, CD73 can catalyse the degradation of NAD rather than its synthesis. Therefore, why reports indicate that CD73 promotes the synthesis of intracellular NAD? The current view is that CD73 increases intracellular NAD levels by mediating the transport of extracellular NAD into cells [33]. Currently, the transmembrane transport protein for NAD is not well understood. The uptake and transport of extracellular NAD remain unknown. However, CD73 may be one of the mechanisms for extracellular NAD uptake and utilization, indirectly promoting intracellular NAD synthesis.

CD73 can dephosphorylate NMN, a metabolite of NAD, into NR. [13]. NR then enters cells through transporters on the cell membrane [34]. The intracellular NR is then phosphorylated to NMN by nicotinamide riboside kinase 1 (NRK1) [35]. Finally, NMN consumes ATP to generate NAD via the catalysis of NMN adenylyltransferase [36]. CD73 failed to increase NAD levels in NRK1 knockout cells, which also confirms the existence of this mechanism [7]. However, contradicting this finding, a study conducted by Wilk et al. concluded that CD73 does not influence intracellular NAD levels. Therefore, the role of CD73 in NAD metabolism and transport remains controversial. Further investigation is required to elucidate the underlying mechanisms.

CD73 indirectly increases intracellular NAD levels by promoting glycolysis

Unlike most normal cells, even under aerobic conditions, tumour cells tend to produce ATP via aerobic glycolysis, which is termed the Warburg effect [37]. The ability of CD73 to promote the tumour Warburg effect by enhancing glycolysis has been demonstrated by Cao et al. [38]. They found that CD73, a hypoxia-responsive gene, was upregulated under hypoxia and promoted the Warburg effect by facilitating glycolysis. CD73-knockdown cells exhibit decreased glycolysis and suppressed tumour cell growth [38]. This effect mainly results from the 5' nucleotidase activity of CD73. The glycolytic level in tumour cells was inhibited after treatment with APCP (a specific inhibitor of CD73 enzymatic activity), while the addition of adenosine restored tumour cell glycolysis [38]. Therefore, the proglycolytic role of CD73 is mediated mainly by regulation of the CD73/adenosine pathway.

Interestingly, glycolysis is beneficial for NAD regeneration and aspartate synthesis. There are two fates of pyruvate in the cytoplasm: one is to produce lactate under the catalysis of lactate dehydrogenase, and the other is to produce acetyl-CoA under the catalysis of pyruvate dehydrogenase and enter the tricarboxylic acid cycle [39]. The former process consumes NADH and promotes NAD regeneration. The latter process converts NAD to NADH during the pyruvate dehydrogenase process. Therefore, when tumour cells metabolize mainly glycolysis, pyruvate is converted to lactate rather than participating in the tricarboxylic acid cycle, which allows the regeneration of NAD in the cytoplasm.

Mitochondrial oxidative respiration and oxidative phosphorylation are the main pathways for NADH consumption and NAD regeneration. This process takes place on the mitochondrial electron transport chain (ETC). The ETC is composed of four complexes arranged in series that are capable of transferring electrons from donors such as NADH to oxygen, consuming NADH to complete the oxidative respiration process [40]. Studies have also shown that the normal function of the ETC is essential for intracellular aspartate synthesis and tumour cell proliferation [41]. However, if tumour cells mainly rely on glycolysis, mitochondrial oxidative respiration is relatively inhibited, and these cells are unable to oxidize NADH to regenerate NAD. This contradicts the promotion of glycolysis to promote NAD regeneration mentioned above. There are some potential explanations. First, although oxidative respiration can consume a large amount of NADH to produce NAD, the tricarboxylic acid cycle itself produces a large amount of NADH for oxidative phosphorylation [42]. Second, the consumption of NADH by the ETC is inhibited by the mitochondrial membrane potential ($\Delta\Psi$) and ATP. In the process of electron transfer, the ETC pumps protons into the membrane space to form an electrochemical gradient inside and outside the cell membrane, which generates $\Delta\Psi$. When the protons in the membrane space pass through the mitochondrial membrane back into the mitochondria, this electrochemical gradient activates FoF1-ATP synthase to catalyse ATP synthesis [43]. However, ATP is not stored in cells and is synthesized only when needed. This effect limits the process of oxidative phosphorylation and NAD regeneration [44]. This conclusion was confirmed in the study of Alba Luengo et al.; that is, the utilization of NADH by the ETC is limited and is regulated by ATP demand. Only when the demand for ATP increases, the ETC will utilize NADH for oxidative phosphorylation to generate sufficient ATP while also regenerating NAD. Since the conversion of pyruvate to lactate catalysed by lactate dehydrogenase can lead to NAD regeneration, tumour cells turn to the glycolytic pathway to produce more NAD when the demand of NAD exceeds that of ATP [45]. This finding also suggested that although tumour cells mainly rely on glycolysis, mitochondrial oxidative respiration is still indispensable. The use of oxidative respiration inhibitors can significantly block the proliferation of tumour cells [46, 47]. In addition, the integrity of mitochondrial DNA (mtDNA) is a necessary condition for tumour growth and proliferation [48].

The CD73-mediated increase in the intracellular NAD level contributes to tumour-promoting effects

After increasing the intracellular NAD level, CD73 exerts its tumour-promoting effects through two pathways. First, it promotes aspartate synthesis by elevating the ratio of intracellular NAD/NADH. Second, it activates NAD-dependent poly (ADP-ribose) polymerase (PARP) to initiate DNA damage repair mechanisms.

CD73 increases the intracellular NAD levels to promote aspartate synthesis

Aspartate is an important substrate for the synthesis of nucleotides and proteins. Therefore, the rapid proliferation of tumour cells inevitably requires a large amount of aspartate as a raw material. A lack of aspartate significantly inhibits tumour proliferation [49, 50]. There are many synthetic pathways for intracellular aspartate [51], and an important one is the two-step synthesis catalysed by malate dehydrogenase (MDH) and glutamate-aspartate transaminase. MDH catalyses the conversion of intracellular malate to oxaloacetate, which is then converted to aspartate by transaminase [52]. In this process, MDH, which catalyses malate dehydrogenase, is regulated by the NAD/NADH ratio. When the level of NAD in cells increases, the activity of MDH increases, initiating the dehydrogenation of malate and the conversion of NAD to NADH. When NAD is deficient, the activity of MDH decreases, thereby inhibiting the synthesis of aspartate. Therefore, changes in the intracellular NAD/NADH ratio affect aspartate synthesis and tumour growth [41].

The conclusion that CD73 promotes aspartate synthesis to promote tumour cell proliferation was confirmed by David et al., who showed that CD73-deficient tumour cells have impaired synthesis of aspartate and impaired proliferation [53]. We speculate that the elevation of intracellular NAD levels by CD73 may be one of the mechanisms through which CD73 promotes aspartate synthesis. As mentioned above, CD73 increases intracellular NAD levels by promoting NAD synthesis, transport, and glycolysis, which may increase the intracellular NAD/NADH ratio. An increase in the NAD/NADH ratio allows MDH activity to be restored, thereby promoting aspartate synthesis [46]. In summary, CD73 directly increases intracellular NAD/NADH levels to compensate for the inhibition of MDH activity caused by NAD depletion, thereby maintaining normal MDH activity to promote aspartate synthesis. However, given the complexity of intracellular NAD and NADH metabolism, it is still inconclusive whether an increase in NAD levels will necessarily result in an increase in the NAD/NADH ratio. Currently, there is no direct evidence indicating that CD73 can elevate the intracellular NAD/NADH ratio. Further research is needed to elucidate the relationship between CD73 and NAD/NADH ratio, as well as aspartate synthesis.

The increase in intracellular NAD levels mediated by CD73 to facilitate aspartate synthesis through the promotion of glycolysis explains the significance of the Warburg effect in promoting tumour growth. Unlike most normal tissue cells, even under aerobic conditions, tumour cells tend to produce ATP via aerobic glycolysis, which is termed the Warburg effect [37]. It is well known that only 2 molecules of ATP are produced when glucose is metabolized to lactate, while 32 molecules of ATP can be generated after complete oxidation of one molecule of glucose, why would tumors choose such an inefficient metabolic way This seems to be controversial. Originally, it was explained that tumour cells have defective mitochondria and are unable to carry out oxidative phosphorylation, thus relying on aerobic glycolysis. However, later studies showed that the mitochondria in most tumour cells are intact [54]. Therefore, the preference for glycolysis in tumour cells is more likely an "active behaviour". Many metabolites in the glycolysis pathway can be used as raw materials for other biosynthesis processes in tumour cells. Proliferating tumour cells need not only an energy supply of ATP but also a supply of molecules for synthesis processes to achieve tumour growth. When the metabolic demand for biosynthesis processes in proliferating tumour cells exceeds the demand for ATP, tumour cells tend to metabolize substances through glycolysis [45]. The mechanism underlying this metabolic transformation in tumor development is complex. As mentioned earlier, CD73 can promote glycolysis, leading to an increase in intracellular NAD levels and the synthesis of aspartate. Therefore, we speculate that when the demand for NAD exceeds that for ATP, CD73 may be one of the key molecules driving this metabolic transformation in tumor cells. Targeting CD73 may potentially disrupt the metabolic conversion in tumor cells, providing an alternative mechanism and direction for CD73-targeted tumor therapy (Fig. 2).

On the one hand, CD73 facilitates the transport of extracellular NAD into cells. First, CD73 promotes the degradation of extracellular NMN, a metabolite of NAD, into NR. Subsequently, NR enters the cytoplasm through transporters on the cell membrane, where it is phosphorylated by NRK1 to generate NMN. NMN is then converted into NAD through two enzymatic steps. On the other hand, CD73 enhances the intracellular NAD/NADH ratio by promoting glycolysis. Pyruvate, under the action of lactate dehydrogenase, is converted into lactate, converting NADH back into NAD and facilitating NAD regeneration, thereby increasing the NAD/NADH ratio. An increase in the intracellular NAD/NADH ratio increases the activity of MDH, promoting the generation of oxaloacetate from malate dehydrogenase and ultimately facilitating aspartate synthesis.

CD73 increases intracellular NAD levels to enhance the DNA damage response

The participation of NAD in the DNA damage response (DDR) is mainly related to the activity of PARP. PARP is a key nuclear protein involved in the DNA damage repair process. When exogenous or endogenous factors cause DNA damage, PARP is activated after binding to DNA

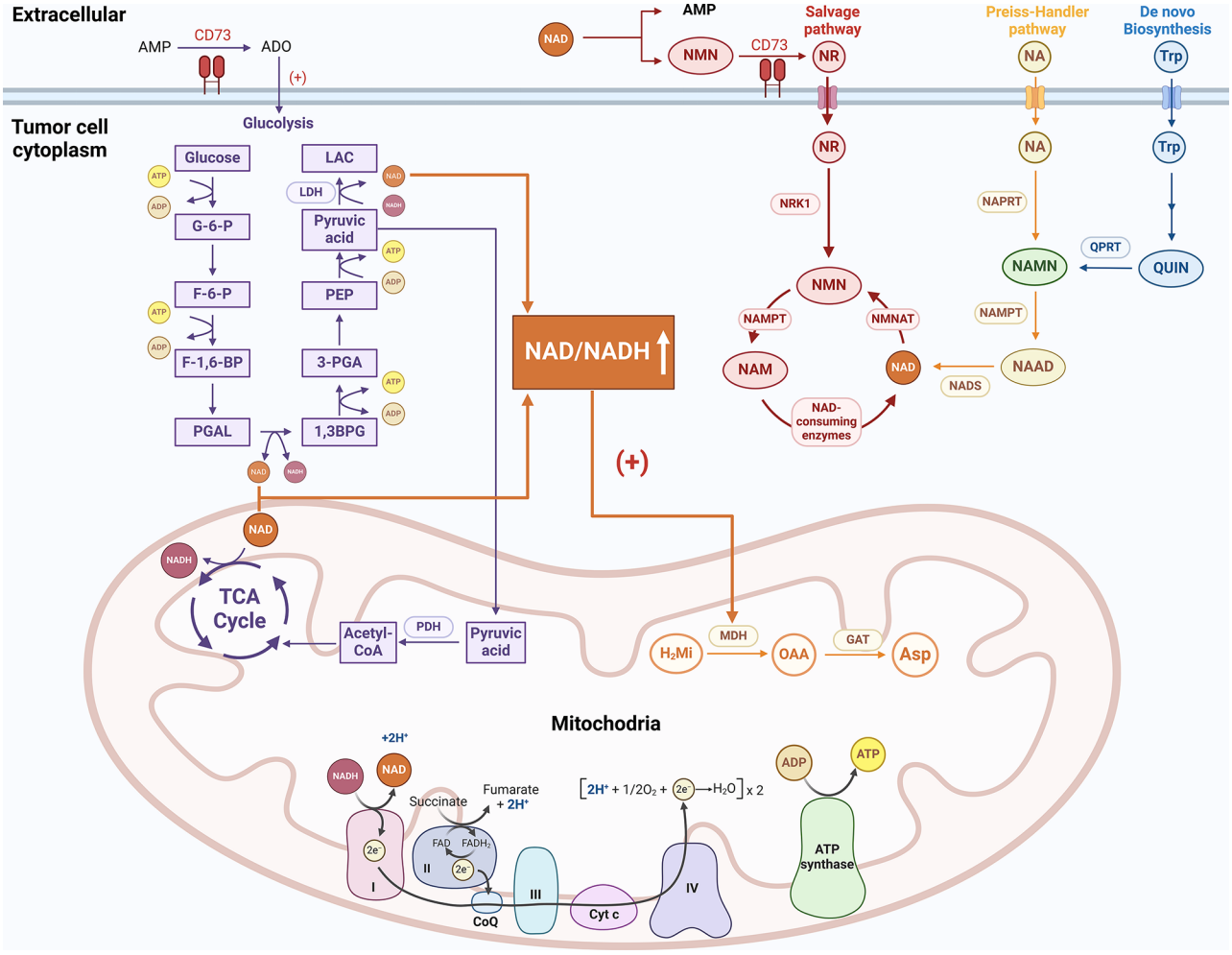

Fig. 2 CD73 increases the intracellular NAD/NADH ratio to promote aspartate synthesis

single-strand breaks (SSBs) and double-strand breaks (DSBs), thereby transferring the ADP-ribose moiety on NAD to the protein receptor to form poly (ADP-ribose) pADPr [55, 56]. Subsequently, pADPr can recruit hundreds of proteins to initiate DNA damage repair [57]. The levels of NAD, a substrate of PARP, greatly affect PARP activity [58]. Studies have shown that CD73 relies on NAD to enhance PARP activity to promote the DDR process. PARP activity is lower in CD73-deficient tumour cells, increasing the sensitivity of these cells to DNA-damaging chemotherapeutics [7]. In addition, the maintenance of the activities of other enzymes involved in the DNA damage repair process, such as SIRT1, SIRT6, and SIRT7, also requires the participation of NAD [59, 60]. Targeted inhibition of NAMPT to block NAD synthesis can increase the sensitivity of breast cancer cells to olaparib (a DNA damage inducer) [61].

On the other hand, CD73 can also prevent cell death and autophagy induced by excessive DNA damage. When DNA damage is excessive, excessive NAD is consumed, which triggers a form of cell death called parthanatos [62]. The underlying mechanism is as follows: First, since many metabolic pathways in cells are NAD dependent, when excess NAD is consumed, cells replenish NAD through other pathways, which leads to ATP depletion and induces necrotic apoptosis [63, 64]. Second, excessive DNA damage leads to the accumulation of pADPr in cells. At this time, the pyrophosphatases NUDIX, NUDT5, and NUDT9 in cells can hydrolyse pADRr to phosphorylated ribose and AMP [65]. This eventually leads to an increase in the intracellular AMP/ATP ratio, putting cells in a state of energy deficiency [66]. This will then activate the AMPK and mTOR signalling pathways to initiate cell autophagy [67]. In summary, CD73 promotes NAD-mediated DNA damage repair by increasing intracellular NAD levels. However, as mentioned above, Wilk et al. revealed that CD73 does not influence intracellular NAD levels. So they conclude that CD73 is not associated with DNA damage repair. The reason for this discrepancy may be attributed to the different cell lines they use. Specific mechanisms underlying this difference requires further investigation.

In addition, the role of the CD73/adenosine pathway in DDR and tumour chemoradioresistance has been confirmed by a large number of studies. CD73 overexpression in human and mouse pancreatic ductal adenocarcinoma cells can prevent gemcitabine- and irradiation-induced DNA damage [68]. In human lung cancer and glioma cells, A2BR signalling can also improve the recovery of radiation-induced DNA damage [69, 70]. The mechanism may be related to several DDR signalling pathways activated in A2RB signalling. First, after binding to adenosine, A2RB can activate protein kinase A (PKA) and protein kinase C (PKC). Among them, PKA can interact with the DNA damage checkpoint (CHK1) to regulate the progression of tumour cell mitosis [71]. PKC can phosphorylate the downstream signalling molecule CHK2 to maintain the stability and integrity of genomic DNA [72]. Second, A2BR signalling can promote epithelial–mesenchymal transition (EMT) by activating the cAMP/PKA and MAPK/ERK signalling pathways [73]. The transcription factor ZEB1, which is activated during EMT, can directly interact with the deubiquitinase ubiquitin-specific protease 7 (USP7) to deubiquitinate CHK1 and initiate DNA damage repair [74].

The DNA damage repair mechanism initiated by CD73 not only gives tumours the ability to resist DNA damage but also inhibits the innate immune response induced by the cGAS-Sting signalling pathway [68]. Due to genomic instability, tumours are prone to DNA damage under internal and external environmental pressures. Damaged DNA fragments then activate the innate immune response by activating the cGAS-Sting signalling pathway, thereby inhibiting tumour growth [75]. The cGAS-sting signalling pathway is mainly mediated by the cytoplasmic DNA sensor cyclic GMP-AMP synthase (cGAS). cGAS can bind to damaged endogenous DNA fragments in the cytoplasm and catalyse the synthesis of the cyclic dinucleotide cyclic GMP-AMP (cGAMP). cGAMP then activates interferon (IFN)-stimulating factor (STING), initiating the transcription of type I interferon (IFN-I) and signal transduction via the NF-κB pathway, thereby activating the innate immune response [76–78]. Therefore, when CD73 increases the stability of the tumour genome, activation of the cGAS-sting signalling pathway is relatively inhibited, thereby inhibiting antitumour innate immunity.

In addition, cGAMP in tumour cells can also be transported into the tumour microenvironment (TME) through transporters on the cell membrane, and cGAMP in the TME can be taken up by other immune cells, thereby activating immune signals [79]. However, the extracellular 5' nucleotidase activity of CD73 can directly induce the degradation of cGAMP in the TME. This process requires the cooperation of ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1). First, ENPP1 can hydrolyse cGAMP released from the cell into AMP, which is then hydrolysed by CD73 into ADO [80]. This converts cGAMP, which has an immunopromoting effect, into ADO, thereby mediating tumour immunosuppression and immune escape [80] (Fig. 3).

When exogenous or endogenous factors cause DNA damage, PARP is activated upon binding to single-strand breaks (SSBs) and double-strand breaks (DSBs) in DNA, transferring ADP-ribose moieties from NAD onto protein acceptors to form poly (ADP-ribose) (pADPr). Subsequently, pADPr recruits hundreds of proteins, initiating the DNA damage repair process. Elevated intracellular NAD promotes PARP-mediated DNA damage repair. CD73-mediated generation of adenosine through AMP hydrolysis also facilitates DNA damage repair processes. CD73-mediated enhancement of DNA damage repair improves the genomic stability of tumours, thereby suppressing the activation of the cGAS-STING signalling pathway and inhibiting the antitumour immune response.

The clinical prospects of targeting CD73 for cancer therapy

Drugs targeting CD73 for clinical cancer therapy

As an important regulatory molecule in cancer, CD73 is highly expressed in most cancers and is closely related to the occurrence, development, and prognosis of cancer [19]. Studies have revealed that in most cancers, such as gastric cancer [81], colorectal cancer [82], hepatocellular carcinoma [11], gallbladder cancer [83], bile duct cancer [84] in the digestive system; non-small cell lung cancer [85] in the respiratory system; and prostate cancer [86] and ovarian cancer [87] in the genitourinary system, tumours with high expression of CD73 often have higher tumour grades, greater invasiveness, and greater lymph node metastasis rates. Therefore, CD73 is an important target for cancer treatment. CD73-targeted therapy has significant clinical potential.

There has been significant progress in the development of drugs targeting CD73 for cancer therapy. Currently, drugs targeting CD73 include CD73 antibodies and CD73 inhibitors. Although most of these drugs are still being tested in preclinical mouse studies and early clinical trials, the results obtained are mostly positive. In early clinical trials, some of the new drugs have shown favorable safety and preliminary efficacy. For instance, in a large clinical randomized phase I trial, results showed that Dalutrafusp alfa (AGEN1423) was well tolerated in advanced solid tumors, demonstrating a positive therapeutic effect and serving as a basis for subsequent clinical combination therapy trials [88]. In addition, results from the large randomized COAST Phase II trial impressively showed that oleclumab in combination with Imfinzi (durvalumab) improved PFS and overall response rate

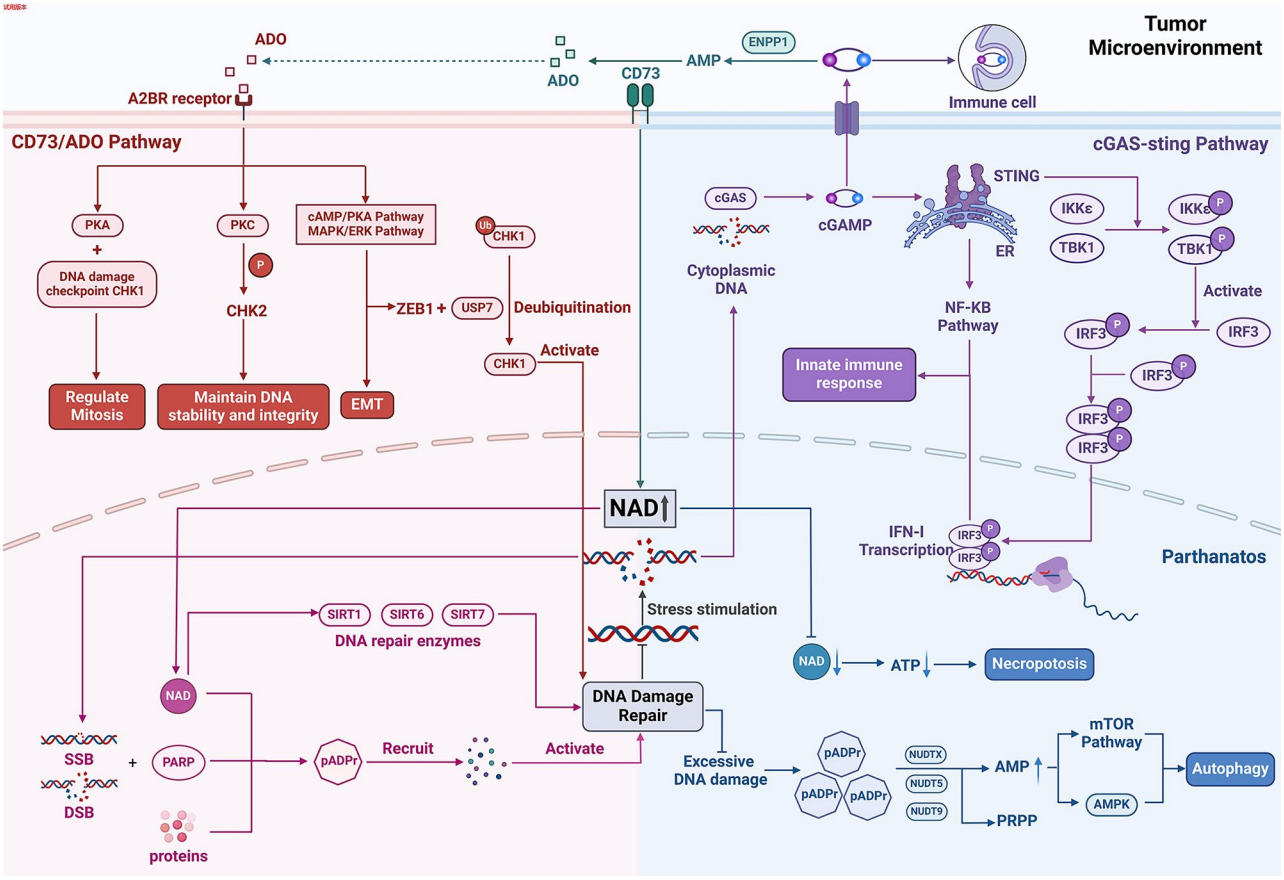

Fig. 3 CD73 promotes DNA damage repair

(ORR) compared to Imfinzi alone stage III non-small cell lung cancer patients and led to the initiation of phase III clinical trial [89].Here, we summarize the clinical trials of drugs targeting CD73, as shown in Table 1.

Targeting CD73 increases radiotherapy sensitivity

Radiotherapy is currently an important clinical treatment for malignant tumours, and endogenous radiation resistance or radiation-induced acquired resistance in tumours seriously affects its efficacy [100]. However, radiation-induced CD73 upregulation may be one of the mechanisms by which radiation-induced acquired resistance arises. Studies have shown that irradiated cancer cells exhibit high CD73 expression [101]. The mechanism of CD73 upregulation by radiotherapy is unclear. Here, we provide several possible mechanisms. First, radiotherapy can induce tumour cells to produce energy through glycolysis rather than aerobic oxidation. This is mainly because the high-energy ionizing radiation produced by radiotherapy generates cytotoxic reactive oxygen species (ROS), such as superoxide anion (O_{2}-.), hydroxyl radical (HO.) and hydrogen peroxide (H_2O_2), which disrupt mitochondrial oxidative phosphorylation [102]. Consequently, tumour cells damaged by radiotherapy often need energy molecules generated by enhanced aerobic glycolysis and the pentose phosphate pathway (PPP) [103]. Alterations in this metabolic pathway leads to the activation of glycolysis and the accumulation of lactate, which promotes CD73 expression by modifying the CD73 promoter through histone lactylation [104]. Second, ROS generated by mitochondrial damage can translocate to the cytoplasm to activate PI3K/Akt signalling in a non-oxygen-dependent manner and promote the transcription of HIF-1α [105]. Elevated levels of the transcription factor for CD73 HIF-1α can directly promote CD73 expression [20]. Third, radiotherapy can induce tumour cell senescence. One of the features of radiotherapy-induced senescence is increased levels of IL-6, which then promotes CD73 expression through the JAK/STAT3 pathway [106].

Fourth, radiotherapy can induce the expression of proinflammatory factors such as IL6 [107] and TGFβ [108] through activation of the nonclassical cGAS-STING signalling pathway. Together with TGF-β1, IL-6 can also induce the expression of CD73 in a variety of cells [109]. Studies have shown that both CD73 and TGF-β are upregulated in all radiation-resistant cells [110], so radiation-induced CD73 upregulation is likely the result

Table 1 Clinical trials of CD73-targeted agents

Compound	Type	Model	NCT No.	Phase	Ref
Oleclumab (MEDI9447)	Anti-CD73 antibody	Non-small-cell lung	NCT05221840	Phase 3 clinical trial	[90]
Oleclumab (MEDI9447)	Anti-CD73 antibody	Non-small-cell lung cancer	NCT03822351	Phase 2 clinical trial	[89]
Dalutrafusp alfa (GS-1423/AGEN1423)	Anti-CD73 antibody	Advanced solid tumour	NCT03954704	Phase 1 clinical trial	[88]
Dalutrafusp alfa (GS-1423/AGEN1423)	Anti-CD73 antibody	Advanced Pancreatic Ductal Adenocarcinoma	NCT05632328	Phase 2 clinical trial	[91]
Mupadolimab	Anti-CD73 antibody	Immune cells in vitro/vivo; Advanced cancer	NCT03454451	Phase 1 clinical trial	[92]
AK119	Anti-CD73 antibody	Advanced solid tumour	NCT04572152	Phase 1 clinical trial	[93]
Bristol-Myers Squibb-986,179	Anti-CD73 antibody	Advanced solid tumour	NCT02754141	Phase 1/2 clinical trial	[94]
IBI325	Anti-CD73 antibody	Advanced solid tumour	NCT05119998	Phase 1 clinical trial	ClinicalTrials.gov
INCA 0186	Anti-CD73 antibody	SCCHN, GI cancer	NCT04989387	Phase 1 clinical trial	ClinicalTrials.gov
IPH5301	Anti-CD73 antibody	Advanced solid tumour	NCT05143970	Phase 1 clinical trial	ClinicalTrials.gov
JAB-BX102	Anti-CD73 antibody	Advanced solid tumour	NCT05174585	Phase 1/2 clinical trial	ClinicalTrials.gov
PT199	Anti-CD73 antibody	Advanced solid tumour	NCT05431270	Phase 1 clinical trial	ClinicalTrials.gov
Sym024	Anti-CD73 antibody	Advanced solid tumour	NCT03311412	Phase 1 clinical trial	[95]
TJ004309	Anti-CD73 antibody	Advanced solid tumour	NCT03835949	Phase 1 clinical trial	[96]
NZV930	Anti-CD73 antibody	Advanced solid tumour	NCT03549000	Phase 1/1b clinical trial	[97]
ORIC-533	CD73 inhibitor	MM patient samples	NCT05227144	Phase 1 clinical trial	[98]
AB680	CD73 inhibitor	Healthy volunteers	NCT03677973	Phase 1 clinical trial	ClinicalTrials.gov
CPI-006	CD73 inhibitor	Advanced solid tumour	NCT03454451	Phase 1 clinical trial	[99]
LY3475070	CD73 inhibitor	Advanced solid tumour	NCT04148937	Phase 1 clinical trial	ClinicalTrials.gov

of overactivation of cytokine TGF-β signalling. The mechanism by which TGF-β promotes CD73 upregulation is likely related to the activity of the deubiquitylating enzyme OTUD4. Prior to transport to the cell membrane but after translation, some CD73 is ubiquitinated. Ubiquitinated CD73 can be recognized by TRIM21 as a degradation signal to control the membrane protein level of CD73 [111]. The TRIM21 protein belongs to the RING-type E3 ubiquitin ligase family, which acts as a potential E3 ligase that can regulate conductive protein hydrolysis [112]. However, the deubiquitinating enzyme OTUD4 can counteract E3 ligase-mediated ubiquitination and thus stabilize CD73 expression [113]. Interestingly, the deubiquitylation of CD73 by OTUD4 is regulated by TGF-β signalling [113]. Therefore, radiotherapy can regulate the deubiquitylation of CD73 by OTUD4 and stabilize CD73 expression by activating the TGF-β signalling pathway.

Radiotherapy induces DNA breaks in tumour cells through high-energy ionizing radiation, triggering the apoptosis pathway and thus effectively killing tumour cells. However, tumour cells may acquire radioresistance through DNA damage repair and apoptosis inhibition [114]. On the one hand, CD73 inhibits apoptosis in tumour cells [115]. On the other hand, CD73 activates the DNA damage response and maintains genomic stability, as described above. Thus, overexpression of CD73 directly inhibits radiation-induced DNA damage and apoptosis, leading to the development of radioresistance. A number of studies have revealed CD73 upregulation in radiation-resistant tumour cells, and inhibition of CD73 reverses radiation resistance [110]. Upregulation of CD73 and downregulation of the apoptotic protein CASP6 were observed in the differential gene expression profile of radiation-resistant oesophageal cancer cells established by continuous fractionated irradiation [116]. Furthermore, in colon cancer cells, combined single-dose anti-CD73 treatment improved tumour sensitivity to radiation [117]. Thus, CD73 upregulation is associated with the acquisition of tumour resistance to radiotherapy. Inhibition of CD73 can increase radiosensitivity and improve the efficacy of radiotherapy.

CD73 as a bridge connecting tumour metabolism and tumour immunity

Tumour metabolism and tumour immunity play crucial roles in regulating the biological behaviour of tumours. CD73 may serve as a bridge connecting tumour metabolism and tumour immunity. First, CD73 exerts dual effects on regulating tumour metabolism and tumour immunity. On the one hand, as an important metabolic enzyme, CD73 can regulate various metabolic processes inside and outside of tumour cells [1]. On the other hand, CD73 regulates the synthesis of metabolic products such as adenosine (ADO) and NAD, which in turn modulate tumour immunity and mediate immune suppression

in the tumour microenvironment. Second, changes in metabolic states can in turn affect tumour immunity through CD73. The Warburg effect in the tumour microenvironment promotes glycolysis and the accumulation of lactate, which then induces the expression of CD73 through lactylation [23], thereby regulating the antitumour immune response. Targeting CD73 can not only enhance the antitumour immune response but also alter the metabolic state of tumours, providing a dual antitumour effect. Therefore, as CD73 represents the intersection between tumour metabolism and tumour immunity, targeting CD73 to combine tumour metabolism therapy with immunotherapy is a promising approach for targeting both tumour metabolism and tumour immunity. Unfortunately, to date, there have been no significant developments in this area. We hope that in the future, drugs targeting CD73 to affect both tumour metabolism and tumour immunity will advance into clinical research, providing new directions for anti-tumour treatment targeting CD73 (Fig. 4).

After radiotherapy, tumour cells upregulate CD73, promoting DNA damage repair and inhibiting apoptosis to develop radiation resistance. Targeted inhibition of CD73 significantly increases tumour radiosensitivity, thereby improving the efficacy of radiotherapy. CD73 not only mediates immune suppression in the tumour microenvironment but also regulates tumour metabolism. Therefore, CD73 has the potential to serve as a bridge between tumour metabolism and tumour immunity, offering new perspectives and directions for clinical research on targeted CD73 therapies.

Unanswered problems

In this review, we elucidated the mechanisms by which CD73 regulates intracellular NAD synthesis and transport. We thoroughly discussed how CD73 impacts the intracellular NAD/NADH ratio, thereby modulating tumour glycolysis and aspartate synthesis and ultimately promoting tumour growth and proliferation. Additionally, we explored how CD73 activates DNA damage repair by elevating intracellular NAD levels, increasing tumour genomic stability, and bolstering tumour adaptability to internal and external stressors, which then suppresses the cGAS-STING signalling pathway, contributing to its protumorigenic effects.

However, there are numerous unresolved questions regarding the role of CD73 in regulating tumour metabolism. Does the role of CD73 in promoting intracellular NAD and aspartate synthesis require the involvement of the adenosine pathway? This point was argued in a study by David et al. This study revealed no significant changes in glycolysis, intracellular NAD, or aspartate levels in tumour cells treated with A2A and A2B adenosine receptor antagonists. Thus, the role of CD73 in promoting metabolic adaptation in tumours is independent of the adenosine pathway [7]. However, the specific mechanism remains to be further investigated. Furthermore, the role of CD73 in promoting intracellular NAD synthesis is currently controversial. A study by Wilk et al. revealed that knockdown of CD73 did not affect intracellular NAD levels and PARP-dependent DNA damage repair [118]. We believe that the reasons for the contradiction in these studies may be the differences in the cell lines they used as well as in the assay methods. The cell line used in Wilk et al.'s study, MCF-7 [118], showed more than tenfold

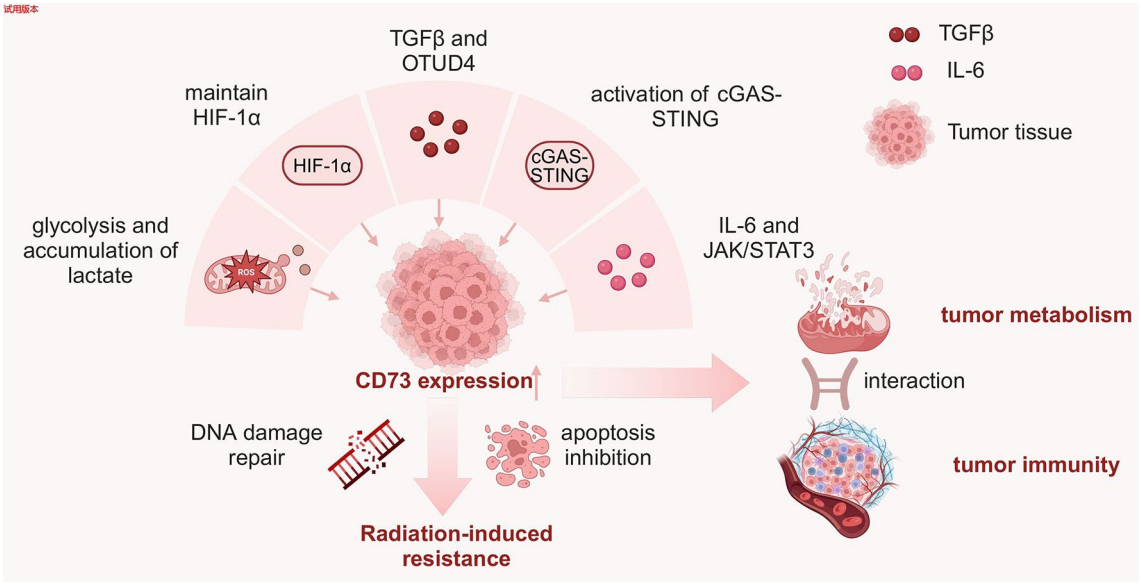

Fig. 4 The clinical prospects of targeting CD73 for cancer therapy

lower expression of CD73 than the MDA-MB-231 cell line used in the study by David et al. [7]. Therefore, in the case of MCF-7 cells with low CD73 expression, knocking out CD73 may have a smaller effect on the cells, leading to the conclusion that CD73 is not related to NAD synthesis or PARP activity.

The relationship between CD73 and glycolysis is not yet known. As mentioned above, in terms of the NAD/NADH ratio, CD73 promotes NAD regeneration and increases the intracellular NAD/NADH ratio, thereby promoting aspartate synthesis. However, the NAD/NADH ratio also regulates the balance between glycolysis and aerobic oxidation by affecting the activities of LDH and pyruvate dehydrogenase (PDH). When the NAD/NADH ratio increases, the activity of LDH decreases, and the activity of PDH increases. Therefore, the metabolism of substances tends to occur via oxidative phosphorylation rather than glycolysis [45]. In this respect, increasing the NAD/NADH ratio of CD73 inhibits glycolysis and the Warburg effect. Wang et al. reported that CD73 inhibits glycolysis. This study revealed that CD73 can inhibit the proliferation of T cells by inhibiting glycolysis through the adenosine pathway [119]. Therefore, it is necessary to further explore the specific mechanism by which CD73 regulates glycolysis to clarify the role of CD73 in tumour cell metabolism and the Warburg effect.

In addition, CD73-deficient mice exhibit decreased levels of serum L-arginine [120]. L-arginine produces NO via a process catalysed by endothelial nitric oxide synthase (eNOS). NO is an important molecule in the regulation of vasodilation and endothelial homeostasis. Since L-arginine is the only substrate for the eNOS-catalysed reaction, a decrease in L-arginine levels severely affects NO production, causing vasoconstriction and decreased permeability, leading to endothelial dysfunction [121]. Interestingly, the metabolism of aspartic acid catalysed by argininosuccinate synthase 1 (ASS1) is an important source of L-arginine [122]. Therefore, we hypothesized that an important reason for the decreased serum L-arginine levels in CD73-deficient mice might be related to the blockage of aspartate synthesis. Unfortunately, no studies have described the relationship between blocked aspartate synthesis due to CD73 deficiency and decreased L-arginine levels.

Although the role of CD73 in tumour metabolism and the specific underlying mechanisms are still unclear, we believe that elucidating the protumorigenic effects of CD73 from a novel perspective distinct from tumour immunity is worth exploring. We hope that this review will provide new research insights into the relationship between CD73 and tumour metabolism, providing novel directions for clinically targeted CD73 therapy and offering hope for cancer patients.

Abbreviations

ASS1	Argininosuccinate synthase 1
ADO	Adenosine
AMP	Adenosine monophosphate
cGAS	Cyclic GMP-AMP synthase
DDR	DNA damage response
DSBs	Double-strand breaks
ENPP1	Ecto-nucleotide pyrophosphatase/phosphodiesterase 1
eNOS	Endothelial nitric oxide synthase
ETC	Electron transport chain
EMT	Epithelial–mesenchymal transition
HIF-1α	Hypoxia inducible factor 1-alpha
mtDNA	Mitochondrial DNA
MDH	Malate dehydrogenase
NAPRT	Nicotinic acid phosphoribosyltransferase
NAAD	Nicotinic acid adenine dinucleotide
NAMPT	Nicotinamide phosphoribosyltransferase
NMNA	TNicotinamide mononucleotide adenylyltransferase
NRK1	Nicotinamide riboside kinase 1
NAD+	Nicotinamide adenine dinucleotide
NAMN	Nicotinamide mononucleotide
PKA	Protein kinase A
PPP	Pentose phosphate pathway
PDH	Pyruvate dehydrogenase
QA	Quinolinic acid
QPRT	Quinolinic acid phosphoribosyltransferase
ROS	Reactive oxygen species
SSBs	Single-strand breaks
Trp	Tryptophan
TME	Tumour microenvironment
USP7	Ubiquitin-specific protease 7
LDH	Lactate dehydrogenase

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Author contributions
E.L. and J.Z. conceived and designed the outline of the article. J.Z., and L.H. prepared the first draft. L.N, W.L and C.S. designed the figures. L.H. and Z.D. prepared the tables. J.Z, L.H, S.L and E.L. carried out the final edits. All authors read and approved the final manuscript.

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Declarations

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