Adenosinergic Axis and Immune Checkpoint Combination Therapy in Tumor:A New Perspective for Immunotherapy Strategy

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April 25, 2022

Abstract

Tumor cells escape anti-tumor immune response in various ways, including functionally shaping the microenvironment through secretion of various chemokines, cytokines, etc. Adenosine is a powerful immunosuppressive metabolite, which is frequently found to be elevated in the extracellular tumor microenvironment (TME). Thus, it has been proposed as a novel antitumor immunoassay to target adenosine generating enzymes such as CD39, CD73, and adenosine receptors in recent years. The discovery of the immune checkpoint such as programmed cell death 1(PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4), have also greatly changed the treatment methods and ideas of malignant tumors. The idea of malignant tumor immunotherapy has been developed from point-to-point therapy targeting immune checkpoint to combine different points of different pathway to create a kind of therapy based on macroscopic immune regulatory system network. This article reviews the theoretical basis of adenosine energy axis and immune checkpoint combined therapy for malignant tumors and the latest advances in malignant tumors.

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The authors declare no potential conflicts of interest.

This work was supported by the National Natural Science Foundation of China Youth Project (grant no. 81900131), the National Natural Science Foundation of China Youth Project (grant no. 8210010129), the Tianjin Municipal Natural Science Foundation (grant no. 18JCQNJC80400), the Tianjin Education Commission Research Project (grant no. 2018KJ043), the Tianjin Education Commission Research Project (grant no. 2018KJ045), and the Tianjin Science and Technology Planning Project (no. 20YFZCSY00060).Tianjin Municipal Health Commission Youth Project(grant no. TJWJ2021QN001);Medjaden Academy & Research Foundation for Young Scientists (Grant No. MJR20221011);Tianjin Key Medical Discipline(Specialty) Construction project.

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Abstract :Tumor cells escape anti-tumor immune response in various ways, including functionally shaping the microenvironment through secretion of various chemokines, cytokines, etc. Adenosine is a powerful immunosuppressive metabolite, which is frequently found to be elevated in the extracellular tumor microenvironment (TME). Thus, it has been proposed as a novel antitumor immunoassay to target adenosine generating enzymes such as CD39, CD73, and adenosine receptors in recent years. The discovery of the immune checkpoint such as programmed cell death 1(PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4), have also greatly changed the treatment methods and ideas of malignant tumors. The idea of malignant tumor immunotherapy has been developed from point-to-point therapy targeting immune checkpoint to combine different points of different pathway to create a kind of therapy based on macroscopic immune regulatory system network. This article reviews the theoretical basis of adenosine energy axis and immune checkpoint combined therapy for malignant tumors and the latest advances in malignant tumors.

Keywords:adenosine, CD39, CD73, A2AR, PD-1, CTAL-4

Adenosinergic Axis and Tumor Immunology

Adenosine, an important regulator of metabolism as well as a key immune checkpoint regulator,which associated with tumors evading the host immune system[1-3].Extracellular adenosine (eADO) is considered as an inhibitor of immune function. One of the major mechanisms of tumor immune evasion is the production of high eADO levels via overexpression of ectonucleotidases[4-6]. An effective immune suppressive microenvironment is sustaind when ADO functions synergistically or in combination with other immunosuppressive mechanisms[7]. In 2006, high extracellular adenosine levels in tumors were discovered to play a key role in evading anti-tumor immune responses [8],the environment that is rich of adenosine in tumor may induce incompetent T cells [1, 9-11].Adenosine pathway is currently considered as an important pathway for the effectiveness of immunotherapy and has become an important target for cancer therapy [1, 12].Endogenous ATP (eATP) can be released in large quantities through cell necrosis, apoptosis and mechanical damage [13],and can also be actively secreted by tumour cells, immunocytes and other histocytes in the TME, triggered by various cell damage factors such as hypoxia, chronic inflammation, cytotoxic drugs, etc. [2].The main source of eADO is from continuous degradation of eATP, which involves many different extracellular enzymes, including NTPDase1/CD39 and CD73[2, 12, 13]. CD39 is found highly expressed in the tumor endothelium of TME and on most immunocytes (including macrophages, myeloid cells and FOXP3+ regulatory T cells (Treg), etc.) surface[3]. CD39 can stabilize FOXP3+Tregs and contribute to its immunosuppressive function [14]; furthermore, CD39 promotes type I Tregs differentiation, produces IL-10, and restricts the activation of NLRP3 inflammatory bodies in dendritic cells (DCs) [2, 12, 13]. CD73 can be found in different kinds of tissues, which includes the colon, liver, kidney, brain, lungs, and heart; leukocytes and endothelial cells of peripheral blood, lymph nodes, spleen and bone marrow[3]. Evidence suggests that CD73 expression and function are elevated in the presence of hypoxia and inflammatory mediators (TGF- B, IFNs, TNF-α, IL-1B and PGE2, etc.), and the expression of CD73 is also increased in several tumor tissues, suggesting that CD73 is involved in tumor genesis and development [2, 12, 13, 15], eATP is decomposed into eADO by the sequence of CD39 and CD73, which binds to adenosine receptors on cell membrane surface [2, 12,

13]. Among several known adenosine receptors, adenosine receptor A2a (A2aR) is the predominant subtype that is mainly expressed in most immunocytes [16]. A2aR stimulation usually provides immunosuppressive signals that inhibit T cell proliferation, cytotoxicity, production of cytokine, NK cell cytotoxicity, production of NKT cell cytokine, upregulation of CD40L, macrophage/DC antigen presentation, etc. [1, 15].(Figure1)

2.Immune Checkpoint

The immune system consists of innate and acquired immunity, which, once activated, clears infectious pathogens and tumor cells. There are inhibitory pathways in antimicrobial or antitumor immune responses that normally maintain autotolerance to avoid excessive damage and limit associated tissue damage [17, 18]. These receptor and ligand inhibitory pathways are known as "Immune Checkpoint" and are used by tumor cells to avoid Immune attack. The development of monoclonal antibodies to inhibit these checkpoints, thereby removing the Inhibition of immunocytes and enabling them to recognize and kill tumor cells, is called "Immune Checkpoint Inhibition". These drugs are called "Immune Checkpoint Inhibitor" (ICI) [19, 20]. FDA-approved anti-CTLA-4 and anti-PD-1 antibodies for cancer treatment led to the belief that immunotherapy for cancer was realistic and further encouraged the development of other new ICIs [21-23]. Immunotherapy is becoming an important treatment for cancer patients [1, 12]. Immune checkpoint blocking (ICB) based on monoclonal antibody (mAb) has also proved to be a safe and effective treatment for hematologic malignancies in the past decade [18, 23]. In oncology, checkpoints currently targeted by inhibitors to amplify the reactivity of T cells, NK cells or bone marrow cells include CTLA-4[24],PD-1,PD-L1(PD1 ligand 1/CD274),LAG-3(CD223),TIM3(T cell immunoglobulin-3),TIGIT(T cell immunoglobulin and ITIM domain)[25],VISTA(V-domain immunoglobulin suppressor of T cell activation)[26],B7/H3(CD276),KIR (killer cell immunoglobulin-like receptors),NKG2A,A2AR,CD39,CD73,CSF1R,CD47,etc.[18, 27, 28].(Figure 2)

Each member of the adenosine signaling pathway constitutes a different drug target, meaning that it is possible for combined therapy with not just only one drug to target this or complementary signaling pathway[29]. Many of these combinations are currently in preclinical and clinical trials, such as anti-CD73 and anti-A2aR combinations, anti-CD73 and anti-PD-1 combinations, as well as anti-A2aR and anti-TIGIT antibody combinations [17, 27, 30-32].(table 1)

3. Combination of CD39 with other immune checkpoints

The rapid development of flow cytometry in recent years has further confirmed the expression of CD39 in tumor cells, particularly in melanoma, lymphoma, and chronic lymphocytic leukemia (CLL) cell lines [12, 13], in melanoma B16F10 mouse model and colorectal cancer Mc-38 mouse model, CD39-defective mice were resistant to tumor metastasis [27, 52]. It has been documented that all cells expressing CD39 exhibit strong ATPase activity, which can be counterbalanced by CD39 inhibitors such as ARL-67156 and POM-1, by measuring the degradation of eATP or release of free phosphate from cell culture supernatant. Treatment with BY40, a CD39 blocking antibody currently under preclinical development, reduced the inhibition of CD4+ and CD8+T cell proliferation, which is induced by tumor tissue, and increased the cytotoxicity that mediated by cytotoxic T lymphocyte (CTL) and NK cell[14]. At present, many studies have proved that human CD39+CD8+ T cells exhibited consistent with draining dysfunction or phenotype gene signature of T cell, including highly expressed inhibitory receptors PD-1 and CTLA-4[53], thus targeted therapy of CD39 combined with other immune binding sites has great significance in the therapy of tumors. Currently, The main CD39 mAb used in clinical and research is IPH5201, which blocks the hydrolysis of ATP by membrane and soluble CD39, thus promoting DC maturation and macrophage activation[10]; BY40 has been reported to block membrane-associated, but insoluble, human CD39 enzyme activity, but its clinical efficacy has not been evaluated [10, 54]; POM1 is mainly used for experimental studies on mice and cell lines [10].

3.1CD39 mAb combined with PD-1 mAb

PD-1 is a gene encoding immunoglobulin superfamily proteins, is focused on sustaining immune tolerance to autoantigens and preventing autoimmune diseases. PD-L1 is a ligand of PD-1. Blocking the interaction between tumor cells expressing PD-L1 and tumor-specific T cells expressing PD-1 using PD-1 or PD-L1 antibodies enhances T cells cytolytic activity[21].It has strong therapeutic value and significance in solid

tumor and hematologic malignancy[29, 55].However, during immunotherapy of tumors, many tumors show resistance to PD-1/PD-L1. One reason many patients exhibit resistance may be due to the immunosuppressive TME, where ROS or nitrogen oxides (NO) released by bone marrow-derived suppressor cells (MDSC) tire T cells and NO longer recognize tumor cells. PD1 resistance and poor prognosis in hepatocellular carcinoma (HCC) patients are associated with up-regulation of CD39 expression in macrophages, and CD39 can be used as a marker of unfavorable prognosis in HCC patients[33].Combination therapy with CD39 mAb significantly improved this situation. It is reported that the therapy combining anti-CD39 and anti-PD1 mAb can further slow tumor growth, and inhibition of CD39 enzyme function can make the tumor model with inherent drug resistance sensitive to PD1 antibody[10, 34].This may be because CD39 mAb and PD-1 mAb could recover the ability of CD8+T cells to produce cytokines. CD39 mAb combined with PD-1 mAb has become one of the targets of many tumor therapies[33].

3.2CD39 mAb combined with CTLA-4 mAb

CTLA-4 is a molecule belonging to the immunoglobulin superfamily first discovered in cDNA libraries of CTLs and expressed in activated T cells, Tregs, and acute myeloid leukemia cells[24, 29].Although CTLA-4 and its homologue CD28 bind to the ligand B7 on B cells and APCs, stimulation of CTLA-4 does not result in T cell activation, but rather to T-cell-mediated antibodies that inhibit and prevent allograft rejection[19, 21].Blocking the CTLA-4-B7 interaction with an anti-CTLA-4 mAb results in an enhanced alloantigen response that inhibits negative signaling to T cells[28].However, anti-CTLA-4 is rarely effective as a single drug in highly oncogenic and immunogenic tumors. Targeting of CD39 with POM-1 has synergistic effect with anti-CTLA-4 checkpoint blocking. Specifically blocking CD39 with POM-1 significantly increased the antitumor activation of CTLA-4 mAb in a mouse model of lung metastasis, and showed better efficacy in a CD39-deficient mouse model of tumor transplanted with B16F10[35]. Recent research also shows that the expression of CTLA-4 and CD39 may be potential target molecules that inhibit Treg activity in situ[36]. Although there is a little literature on the combination of CD39 mAb with CTLA-4 mAb, according to the current study, the combination of the two mAb has great potential in tumor therapy, especially in the treatment of tumor metastasis.

3.3CD39 mAb combined with TIGIT mAb

TIGIT is an inhibitory receptor and is expressed on lymphocytes, it has recently attracted attention as the latest target for tumor immunotherapy. It shows interplay between TIGHT and CD155, which is expressed on APCs or tumor cells, reducing T and NK cell function. TIGIT, a significant inhibitor of antitumor response, blocks the tumor immune cycle in multiple steps[56-58]. Several studies have shown that blocking TIGIT can prevent various solid tumors and hematologic malignancies. In AML, inhibition of CD39 combined with TIGIT can apparently increase AML cell lysis in 2/3 cell lines, and combined inhibition of TIGIT and CD39 significantly improved NK cell killing activity in vitro, thus further enhancing NK cell killing effect on AML cells[37, 38, 59].Due to the difference in the expression of the TIGIT/PVRIG axis and CD39 in different NK cell subsets, joint blocking of these pathways may enhance the cytotoxic function of different NK cell subsets in vivo. In addition, it has been preliminarily proved that RORγ agonists can simultaneously reduce the expression of CD39, TIGIT and other immune checkpoints on lymphocytes, and integrate multiple anti-tumor mechanisms into one therapy, which can not only enhance immune activity, but also reduce immunosuppression, thus effectively inhibiting tumor growth[60].

4.Association of CD73 with other immune checkpoints

CD73 is expressed in various kinds of cancer, promoting tumor growth, metastasis, and drug tolerance in glioblastoma, melanoma, leukemia, colon, breast, ovarian, and bladder cancers[12, 15, 61].In human breast cancer cells, high expression of CD73 is related to low response and high resistance to anthracyclines[40, 42, 62].High level of CD73 is related to immunosuppression and tumor progression. Overexpression of CD73 in tumors not only leads to metastasis of tumor cells and anthracyclines resistance, but also leads to immune escape because of excess of adenosine production[63, 64].Therefore, inhibitors of CD73 are currently used in combination with existing cancer therapies for cancer immunotherapy, including anti-PD-1/PD-L1 and anti-

CTLA-4 therapies[40, 42, 63].Although blocking CD73 alone does not result in a cure, inhibition of CD73 increases the antitumor effect of blocking immune checkpoint therapies, including anti-CTLA-4 and anti-PD-1 [15, 20, 63].Synergistic effects of combined CTLA-4 mAb with CD73 mAb and combined PD-1 mAb with CD73 mAb immunotherapy have been observed in both preclinical models of breast cancer and colon cancer[20, 41, 64].Currently, MEDI9447(AstrazenecaMedimmune), a human IgG1CD73 mAb[42], can selectively inhibit the activity of CD73ECN, and can cross-react with mouse and human CD73. MEDI9447 internalized the desetting of CD73 from the cell surface, thereby inhibiting the conversion of AMP to adenosine and removing inhibition of T cell proliferation that is mediated by AMP. In an immunoactive mouse tumor model, MEDI9447 reduces immunosuppressive effects and promotes antineoplastic function[41];BMS986179, a high affinity antibody, inhibits the activity of CD73 and mediates the internalization of CD73[15, 65];CPI-006 (also known as CPX006) acts mainly by inhibiting CD73 activity and/or inducing CD73 downregulation; IPH5301, which blocks AMP from degrading to the immunosuppressant adenosine. At present, these antibodies are undergoing early-stage clinical trials [10, 66] ◦

4.1CD73 mAb combined with PD-1/PD-L1 mAb

As is mentioned above, CD73-derived adenosine strongly mediates tumor immune status and metastasis, and weak patient response to PD-1 antibodies may also be associated with elevated intratomatous adenosine levels. In this context, the combination of CD73 mAb and PD-1 mAb may be particularly effective in the immunotherapy of tumors. Currently, various studies are focusing on the clinical effect of combining treatment of CD73 inhibition with PD-1 blockade. In melanoma, breast cancer, colon cancer, non-small cell lung cancer (NSCLC), prostate cancer and other malignant tumors[20, 39, 41], Combining CD73 mAb with PD-1 mAb has shown a more significant effect than these drugs alone. The high expression of CD73 on the surface of tumor cells shows a weaker effect of immunotherapy with PD-1 antibody, and the combined use of PD-1 mAb and CD73 mAb prominently inhibited tumor growth[42],increased gene expression related to inflammation and T cell function, causing an increase in the number and activity of tumor-infiltrating CD8+T cells and the production of IFN-γ and TNF-α of them[16, 42].It is also reported that MEDI9447, when combined with anti-PD-1 antibodies, can produce a better antitumor effect, which is supported by multiple phase I/II trials based on MEDI9447. Preliminary phase I data for MEDI9447(NCT02503774) have recently been reported[20].The safety of MEDI9447 and duvacizumAb (anti-PD-L1) treatment is controllable, and PD-1 is consistent with its mechanism of function. BMS-986179 was also found to enhance the antineoplastic activity of anti-PD-1mAb in preclinical animal models[15, 67].Interestingly, the combination of A2AR antagonist and PD-1 antibody also showed an anti-metastasis effect. However, the combination therapy with A2AR antagonists was effective only when tumors expressed high CD73 levels, suggesting that CD73 can also be used as a potential tumor indicator to assess patients' benefit from combination therapy and prognosis[12, 15, 40].

4.2CD73 mAb combined with CTLA-4 mAb

The combination of CD73 mAb and PD-1 mAb was found to be more effective against both subcutaneous and metastatic tumors than CD73 mAb and CTLA-4 mAb[20].This may be due to the stronger antineoplastic activity of PD-1 mAb itself than CTLA-4 mAb, or the synergistic effect of CD73 mAb and PD-1 mAb on Tregs. However, CD73 mAb combined with CTLA-4 mAb still has clinical significance that cannot be ignored. CD73 mAb combined with CTLA-4 mAb significantly improved median survival in tumor metastasis model of mice[67].In melanoma, the efficacy of anti-CTLA-4 therapy can be enhanced by targeting various immunosuppressive mechanisms in tumor tissue, including CD73. CD73 antibody combined with CTLA-4 mAb has a significant inhibitory effect on melanoma growth. In the melanoma model of mice, the percentage of infiltrated CD8+T and CD4+T cells was particularly increased after the combination of the two antibodies, and the proportion of Tregs was also increased compared with that of the two antibodies alone, which may be due to the increase of CD4+T cells after the combination of the two monoclonal antibodies. At the same time, IFN -γ levels were increased in melanoma tissues of mice treated with CD73 antibody in combination with CTLA-4 antibody[43],CTLA-4 mAb is also of great clinical significance in hematologic malignancies such as AML and MDS[18],which has great potential in combination with CD73 antibody in the treatment

of blood malignant diseases.

5.Association of A2AR with other immune checkpoints

A2a receptor (A2aR) in the pathway of adenosinergic axis is becoming an important immune checkpoint. Adenosine levels in the extracellular fluid are upgraded in the TME due to the special metabolism of tumor cells, which contributes to tumor immune escape. Therefore, inhibitors of A2aR are being sought to enhance the effect of immunotherapy[2, 12].Several A2aR antagonists have been developed at present and have been tested in multiple preclinical studies. At least four drugs are currently in phase I clinical trials: CPI-444[9] (Corvus), PBF-509 (Novartis/Pablobiofarma), MK-3814 (Merck), AZD4635 (AstraZeneca/Heptares)[12].CPI-444 has been reported to intensify antineoplastic immunity and enhanced anti-PD-L1 mAb activity in mice. CPI-444 has also been exhibited to intensify the antitumor effect of adoptive metastases of HER2-specific CD8+T cells in tumor-bearing mice treated with cyclophosphamide and a novel gene-expressed whole cell vaccine (GVAX)[9]. Vipatant (REDOX /Juno therapy) and Etradine (Kyowa Hakko Kirin) are other kinds of promised oral A2a antagonists that have previously had an exam in clinical trials in Parkinson's disease and may be effective in cancer patients. Recent research shows that A2aR restrains T cell proliferation and cytokine secretion and increases expression of PD-1 and CTLA-4 on surface[68, 69].Currently, the combination therapy of A2aR and other immune checkpoints is also attracting attention[9, 12].

5.1A2aR mAb combined with PD-1/PD-L1 mAb

Since the expression of A2a is increased on antigen-activated T cells and PD-1 is involved in inhibiting T cells function, the combination of targeted blocking of these two molecules is considered as a new direction for tumor therapy. Recent clinical researches show in RCC (renal cell cancer) patients, the A2AR and PD-L1 expression in the primary tumors may foresee the consequences of therapy with anti-VEGF agents and ICIs[47], the A2AR antagonist Ciforadenant, showed monotherapy activity in patients who are resistant to or intractable to previous anti-PD-L1 therapy. Though this trial did not compare the effects of monotherapy with combination therapy deliberately, treatment with A2AR antagonist plus anti-PD-L1 appeared to improve efficacy[46]. Studies have shown that blocking A2aR with CPI-444 reduces the expression of checkpoints of various pathways on T-effs and Tregs, including PD-1 and LAG-3. By reducing the expression of immune checkpoints on these T cells, the threshold of anti-PD-1 treatment is lowered. That is, the synergistic reaction of CPI-444 combined with PD-1 mAb therapy[9, 44]. Moreover, A2aR blocker significantly reduced the expression of PD-1 and LAG-3 in draining lymph nodes of mice which were tumor-bearing [49]. Another group successfully combined A2aR blockers with anti-PD-1 inhibitors in an anti-tumor regimen in a mouse model[31]. Mittal et al.[45] also reported that uniting SCH58261, the A2aR inhibitor, with anti-PD-1 therapy significantly reduced the burden of metastasis compared with either monotherapy alone. Uniting therapy with PD-1 mAbs and CPI-444 showed significant improvement in tumor regression and survival in tumor models of CT26 and MC38(more significant in CT26 tumor models)[44].In NSCLC mouse models, A2a receptor inhibition overcomes resistance of tumor cell to PD-1/PD-L1 blocking treatment. Meanwhile, A2AR and CD73 were up-regulated in mice treated with PD-1 mAbs or PD-L1 mAbs[48, 49].In mouse models of breast, colon, and hepatocellular carcinoma, drug resistance of tumor cells to PD-1/PD-L1 can be prevented by dual blocking of PD-1 and A2aR. Blocking A2aR after virus attack also reduced the expression of PD-1, LAG-3, and TIM-3 on the cover of CD8+T cells and Tregs. These abundant in vivo and in vitro experiments suggest that the combination of A2aR blocker and PD-1/PD-L1 antibody is of great significance in the treatment of tumors in the clinical[30, 67].

5.2A2aR mAb combined with CTLA-4 mAb

Combining A2aR mAb CPI-444 with anti-CTLA-4 therapy eliminated tumors in up to 90 percent of treated mice, including restoring an immune response in a model against an incomplete response to CTLA-4 monotherapy. Moreover, tumor cells remained suppressed after reinoculated mice with tumor cells, suggesting that CPI-444 induces systemic antineoplastic immune memory, and that combination of CPI-444 with CTLA-4 mAb increases the presence of CD8+T cells and IFNγ and Gzm B levels in tumors[67].In a

melanoma model of mice, inhibiting both CD73 and A2aR increased CTLA-4 blockade therapeutic effect. Blocking A2aR plays an important role in regulating function of T cells and significantly reduces melanoma growth[43].Most importantly, the combination of A2aR antagonists and anti-CTLA-4 therapy significantly restricted tumor growth and enhanced anti-tumor immune response[9, 67]. Additionally, other studies have shown that the concomitant blocking of A2aR and CTLA-4 in T cells can synergistically enhance the anti-tumor response through downregulating PKA, SHP2 and PP2Aα signaling pathways, providing theoretical basis for A2aR mAb combined with CTLA-4 mAb as a new treatment regimen for tumors[50].

5.3A2aR mAb combined with TIGIT mAb

The frequency of TIGIT^{+}NK cells in patients' blood was negatively related to the prognosis of AML. Compared with healthy subjects, AML patients had abnormal NK cell populations in peripheral blood (PB) and bone marrow (BM), which were shown as increased frequency of TIGIT+, PVRIG+, CD39+ and CD69+NK cells. This makes TIGIT a target for AML treatment[38]. Purinergic pathway also regulate the function of NK cells. Proliferation and hypoxia of tumor cells increase the utilization of ATP and activate cancer-related CD39 and CD73, which catalyze the continuous dephosphorylation of ATP to AMP and then to eADO. Extracellular adenosine accumulation interacts with adenosine receptors expressed on the surface of NK cells and inhibits signaling through A2aR, so A2aR antibodies are also an important target for tumor therapy[51]. It has been demonstrated that combined blocking of TIGIT and A2aR could enhance NK-92 cell-mediated cytotoxicity in AML[38]. In other tumors, the combination of TIGIT mAb and A2aR mAb is still being explored[51].

6.Conclusion

In recent years, therapeutic advances in cancer immunotherapy (CIT) have emerged rapidly, reflecting the importance of human immune system interactions with cancer, as well as the complex and highly regulated nature of the immune system[70, 71]. Under the background of complex immune network, point-to-point therapy has been unable to achieve satisfactory tumor treatment effect, so combining various targeting axes or immune checkpoints will become a new direction of tumor treatment.

Adenosine axis's role in tumor microenvironment is mainly induced by hypoxia, so some studies have also called it hypoxia-adenosine axis. Extracellular adenosine increases in hypoxic conditions, and simultaneously the expression of CD39 and CD73 improves simultaneously[7]. Antihypoxia-adenosine therapy is synergistic with other immune checkpoint inhibitors, such as CTLA-4 mAb and PD-1 mAb. The combination of anti-hypoxia-adenosine strategies may enhance clinical response to other immunotherapies and chemotherapy and radiotherapy[67].With advances in the treatment of tumors with immune checkpoint blockers such as CTLA-4 and PD-1/PDL1, more therapeutic targets have been sought, including but not limited to the immune targets in the adenosine energy axis mentioned above, in order to overcome the problems of incomplete tumor regression or recurrence after treatment[3].More identified immune checkpoint suppressor molecules are emerging as new potential targets for tumor therapy. In the TME, these molecular mechanisms may operate and may be supplementary to immunotherapies that had been approved [22, 72, 73].When immune escape of tumor cells becomes a difficult problem to conquer tumors[74],the use of these immune checkpoint inhibitors can offset immune escape of tumor cells to a certain extent and further improve the response rate. Without increasing or even decreasing the adverse events related to excessive damage of tissue, autoimmunity, and other immune-associated side reactions associated with the use of immune checkpoint inhibitors alone[18, 75-77].In the future, the treatment trend of malignant tumors will be developed from point-to-point therapy targeting individual immune checkpoints to the combination of immune networks composed of various signaling pathways, such as adenosine axis. Even therapies that are not traditionally considered immunotherapies can induce or enhance antitumor immunity. As a result, they may force tumors to upregulate immune checkpoints ,which can be blocked as part of a combined strategy[76, 78-81].

7. Acknowledgments

This work was supported by the National Natural Science Foundation of China Youth Project (grant no. 81900131), the National Natural Science Foundation of China Youth Project (grant no. 8210010129), the

Tianjin Municipal Natural Science Foundation (grant no. 18JCQNJC80400), the Tianjin Education Commission Research Project (grant no. 2018KJ043), the Tianjin Education Commission Research Project (grant no. 2018KJ045), and the Tianjin Science and Technology Planning Project (no. 20YFZCSY00060).Tianjin Municipal Health Commission Youth Project(grant no. TJWJ2021QN001);Medjaden Academy & Research Foundation for Young Scientists (Grant No. MJR20221011);Tianjin Key Medical Discipline(Specialty) Construction project.

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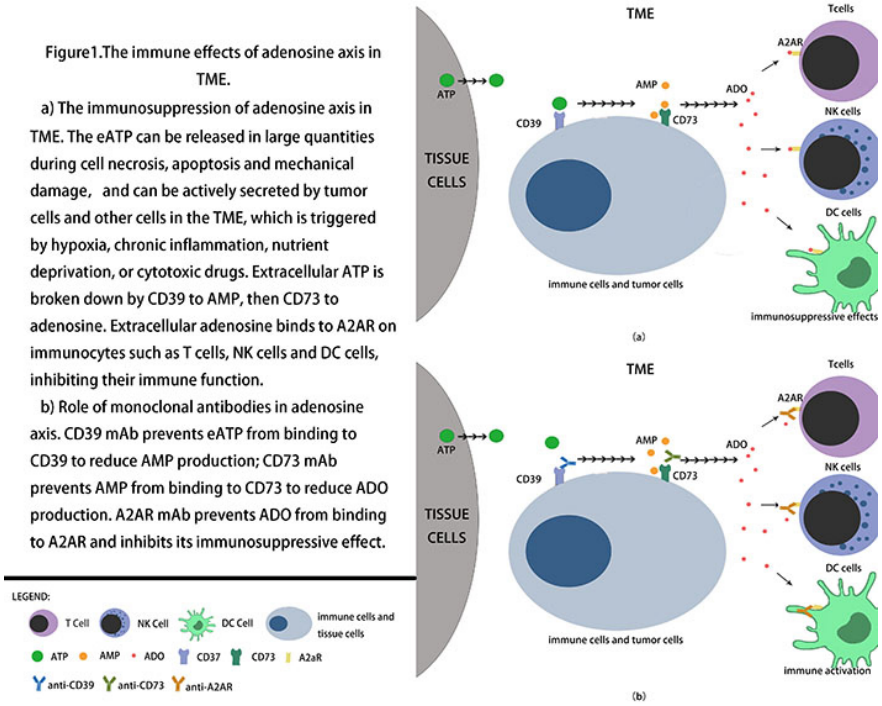

Figure1.The immune effects of adenosine axis in TME.

a) The immunosuppression of adenosine axis in TME. The eATP can be released in large quantities during cell necrosis, apoptosis and mechanical damage, and can be actively secreted by tumor cells and other cells in the TME, which is triggered by hypoxia, chronic inflammation, nutrient deprivation, or cytotoxic drugs. Extracellular ATP is broken down by CD39 to AMP, then CD73 to adenosine. Extracellular adenosine binds to A2AR on immunocytes such as T cells, NK cells and DC cells, inhibiting their immune function.

b) Role of monoclonal antibodies in adenosine axis. CD39 mAb prevents eATP from binding to CD39 to reduce AMP production; CD73 mAb prevents AMP from binding to CD73 to reduce ADO production. A2AR mAb prevents ADO from binding to A2AR and inhibits its immunosuppressive effect.

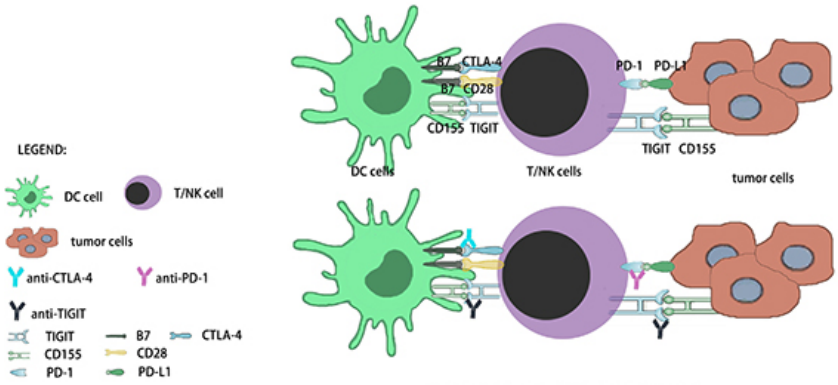

Figure2. Immune checkpoints in current studies

i) The PD-1 is expressed on activated T cells in the early-stage lymph node as well as late-stage tumor tissues in the TME. In the early stage and late stage, PD-1 sustains immune homeostasis by decreasing activated T cells function. Tumors might become resistant to this suppression signaling, increasing the survival potential of the tumor cells. ii)The CTLA-4 is expressed on T cells that are activated by DCs in the lymph node. By MHC interaction with T cell receptor and B7 signal interaction with CD28 on T cells. In order to sustain immune homeostasis, CTLA-4 downregulates the function of activated T cells through the interaction of B7 signaling with CTLA-4 on T cells. Tumors may develop toleration to this inhibitory signal, thus improving the survival potential of tumor cells. iii) TIGIT is expressed both on NK cells and T cells, which includes CD4+ T cells, CD8+ T cells and Tregs. TIGIT has three ligands, CD155, CD112 and CD113, and the main ligand for TIGIT is CD155. The main effect of TIGIT is downregulating the function of NK cells and T cells.

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