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Review

# Adenosine signaling: Next checkpoint for gastric cancer immunotherapy?

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ABSTRACT

Adenosine (ADO), generated by the ectonucleotidase CD39 and CD73 from ATP, interacts with its specific G protein-coupled receptors, which can impair anti-tumor immune responses inhibiting the infiltration and function of $CD8^+$ T cell and natural killer cell. Recent studies have also identified that ADO pathway plays a critical role in tumor immune surveillance, especially for some non-solid cancers. In addition, although immune checkpoint therapy targeting ADO pathway in gastric cancer is still in an early phase, encouraging results have come out from some drugs targeting ADO pathway. Therefore, target ADO signaling may be a new promising strategy to treat gastric cancer. In this review, we summarized recent works on the role of ADO in cancer immunotherapy and also discussed relative mechanisms underlying the function of ADO signaling in cancer immune responses.

## 1. Introduction

Gastric cancer is the fourth most frequent type of cancer in the world. Although the incidence of gastric cancer declines in the last few decades, current estimated mortality for gastric cancer still reaches about 738,000 worldwide [1, 2]. Surgery and systemic chemotherapy are the most common treatment for gastric cancer. However, majority of patients with gastric are diagnosed at a relatively late stage, which largely limit the efficacy of surgery in gastric cancer treatment. In addition, although recent advance in individualized chemotherapy scheme has benefited gastric cancer patients, the inevitable side effects are still a challenge for patients [3, 4]. The success of cancer immunotherapy during the past several years has highlighted the potential to utilize a patient's immune system to eradicate cancer [5]. Immune checkpoint blockade therapy, such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), programmed cell death protein 1 (PD-1), growth factor receptor-2 (VEGFR-2), and epidermal growth factor receptor-2 (HER2), have been employed in cancer therapy, with excellent and durable responses achieved in many kinds of malignancies including melanoma, squamous non–small cell lung cancer, lymphoma and renal cell carcinoma [3, 6–8]. However, limited successful responses are reported in most solid tumors such as gastric cancer and colorectal cancer.

Adenosine (ADO), is an endogenous purine nucleoside composed of an adenine attached to a ribose sugar molecule moiety. The biological function of ADO has been intensively investigated in the last decade [9]. Through binding to four G-protein-coupled receptors (GPCRs), ADO plays multiple regulatory roles in many pathological and physiological processes such as inflammation, hypoxia, ischemia trauma, autoimmune diseases, neoplastic milieu and even pulmonary arterial hypertension [10–13]. It is suggested that ADO can be generated by the ectonucleotidase CD39 and CD73 from ATP under hypoxic tumor microenvironment (TME), and can further deaminate to inosine by adenosine deaminase, which can impair anti-tumor immune responses by inhibiting the infiltration and function of $CD8^+$ T cell and natural killer cell (NK) [14–16]. Based on these facts, some drugs targeting ADO pathway are tested and now are under preclinical evaluation with encouraging results emerged [17]. Thus, ADO signaling pathway may be a promising checkpoint for gastric cancer immunotherapy.

In this review, we sought to summarize recent findings on the function of ADO signaling in immune regulation of tumors, and also discussed the mechanisms through which ADO signaling resulted in gastric cancer immune responses.

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## 2. Adenosine and its receptors

Adenosine is an endogenous purine nucleoside, composed of an adenine attached to a ribose sugar molecule moiety. ADO modulates different physiological function in all the organs, tissues and cells of body [9, 18]. The levels of extracellular ADO, estimated to be around 1 μM under normal conditions, can increase up to 100-fold in situations of ischemia, trauma, hypoxia, inflammation, or tumor [19, 20]. The Warburg effect is an important metabolic feature in many types of cancer [21]. In hypoxia TME, deprivation of nutrients or oxygen limits the availability of energy sources and induces the accumulation of extracellular adenosine 5′-triphosphate (ATP), leading to an increase of ADO levels in tumours [22]. Once synthesized, ADO is transferred to the extracellular space by two kinds of nucleoside transporters, concentrative nucleoside transporters (CNTs) and equilibrative nucleoside transporters (ENT1, ENT2, ENT3 and ENT4) [23, 24].

The function of ADO depends on receptor-dependent and -independent mechanisms [25]. ADO receptors, belonging to the class A (rhodopsin-like) G protein-coupled receptor (GPCR) superfamily, are classified into A1, A2A, A2B, and A3 groups based on their pharmacologic and functional characteristics [26]. A1 and A3 receptors are paired with Gi signal transduction proteins or ion channels to inhibit adenylate cyclase and cyclic AMP(cAMP) production, while A2A(high-affinity) and A2B (low-affinity) receptors are coupled with G proteins Gs or Gq and activates adenylyl cyclase or phospholipase C [27, 28].

The expression of ADO receptors are regulated by several pro-inflammatory cytokines. Interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) can increase A2A receptor expression on human monocytes THP-1 and enhance receptor function [29]. By contrast, IFN-γ reduces the expression of A2A receptors by downregulating the expression of adenylyl cyclase [30]. Proinflammatory stimuli also regulate A2B receptor expression in macrophage, and enhance their sensitivity to immunosuppressive extracellular ADO [31]. On the other hand, ADO itself can also regulate its receptor expression through heme oxygenase-1(HO-1)-dependent mechanism [32].

## 3. CD39-CD73 axis

ADO is catalyzed from ATP in the sequential steps mediated by two ectonucleotidases, CD39 and CD73 [33]. CD39, also known as ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1), is one of the first eight NTPDase enzymes that has been cloned and sequenced [34]. It can hydrolyze the extracellular ATP and adenosine diphosphate (ADP) into adenosine monophosphate (AMP) [35, 36]. CD39 is abundantly expressed in numerous cells, including regulatory T cells (Treg), leukocytes, and endothelial cells [37–39]. CD73 is a GPI-anchored enzyme that can hydrolyze AMP into ADO and inorganic phosphate,serving as rate-limiting step of ADO synthesis [40]. CD73 has been identified to be expressed on various types of cells including cancer cells and tumor microenvironment infiltrating immune suppressive cells such as Tregs and myeloid-derived suppressor cells (MDSCs) [41]. CD73 has been identified as a critical check point in regulating the duration and the magnitude of the“adenosine halo” surrounding immune cells [42]. In addition to its enzymatic function, CD73 also mediates cancer cell invasion and progression through inhibiting anti-tumor immune response of the host immune system [43].

Due to the key role of ADO mediated immune evasion, dysregulation of CD39 and CD73 has been associated with multiple human cancers development [44–46]. Up-regulation of CD73 in TME is associated with highly invasive cancer phenotype, drug resistance, displaying tumor-promoting effects [47]. Overexpression of CD39 and high tumoral $CD39^+/CD8^+$ ratio has been verified to be associated with adverse prognosis in resectable gastric cancer [48]. Similarly, expression of CD73 is positively correlated with tumor stage and is associated with poor prognosis in head and neck cancer (HNSCC) [49], ovarian cancer [50], breast cancer [51], and B Lymphoblastic Leukemia [52]. Therefore, CD73 has also been proposed as a potential prognostic and diagnostic marker in certain malignancies such as prostate cancer and ovarian cancer [50, 53].

The potential of blocking CD39-CD73 axis-mediated immunosuppression in cancer immunotherapy is being actively explored [54–58]. CD39 expression on Tregs may inhibit NK activity and is permissive for melanoma metastatic growth [59]. Mittal *et al.*…, showed that combined therapy with anti-CD73 and anti-PD-1 mAb can reduce the tumor metastatic burden in implanted and spontaneous models of melanoma and breast cancer [60]. Li *et al* also found that down-regulating the expression and decreasing ectoenzymatic activity of CD39 and CD73 by metformin blocked the suppressive function of MDSC in patients with ovarian cancer [61]. In addition, CD73 is also identified to mediate tumor resistance to existing treatment such as chemotherapy, radiotherapy and molecular targeting therapy in many kinds of carcinomas [62–65].

Because of the key role of CD39-CD73 axis in ADO synthesis, the regulatory mechanism for CD39-CD73 axis on tumor development is another hotspot. Clément Barjon *et al.* demonstrated that IL-21 favored the emergence of a subpopulation of $V_\gamma 9V\delta 2$ T cells that expressed the ectonucleotidase CD73, which inhibited T cell proliferation in a CD73/ADO-dependent manner [66]. Reduced CD73 expression by IL-1β can overcome the ATP mediated suppression on IFN-γ production by T cells [67], while activation of MAPK mutations and growth factors during immunotherapy may promote CD73 expression, leading to both nascent and full activation of a mesenchymal-like melanoma cell state program [68].

## 4. ADO in tumor immune regulation

Why tumors and antitumor immune cells can coexist in a “hostile” tumor microenvironment is poorly understood for a long period [69]. Emerging evidence suggested that ADO and other components of the ADO pathway play a non-redundant role in tumor immune escape [70]. ADO is a crucial immunosuppressive metabolite, necessary for protecting against an overzealous immune reaction during inflammation and is also indispensable for the recovery from tissue damage [71]. In hypoxia TME, tumor cells adapt to this condition by activating aerobic glycolysis. At the same time, deprivation of oxygen limits the availability of energy sources and induces the accumulation of extracellular ADO in tumors [72]. (See Fig. 1.)

By engaging ADORs, ADO activates (A2A, A2B) or inactivates (A1, A3) adenylyl cyclase (AC), and modulates the levels and activity of 3′5′-cAMP in effector T cells [73]. cAMP is an intracellular second messenger involved in numerous cellular functions, leading to different biological outcome [74]. Furthermore, accumulation of intracellular cAMP induces protein kinase A-mediated phosphorylation and activation of COOH-terminal Src kinase (Csk) [75]. Csk then may phosphorylate and inhibit lymphocyte-specific protein tyrosine kinase (Lck), produce a broad range of immunosuppressive effects, such as diminishing immune active cytokines (e.g., IFN-ϒ and IL-12), increasing production of immunosuppressive cytokines (e.g., TGF-beta, IL-10), upregulating of alternate immune checkpoint pathway receptors (e.g., PD-1, LAG-3), and/or reducing levels of major histocompatibility complex (MHC) II expression on DC, all of which result in less effective T-cell activation and decreased anti-tumor immune response [76, 77].

In the context of cancer, the accumulation of extra-cellular ADO in tumors suppresses antitumor immune responses, essentially via the activation of A2A adenosine receptors [78, 79]. The mechanisms of ADO-rich TME in obtaining a broad spectrum of strong immunosuppressive properties include the following aspects: 1) Stimulation of A2AR impairs activities of CTL cells (proliferation, cytokine production, and cytotoxicity), NK cells (cytotoxicity), NKT cells (cytokine production and CD40L upregulation), macrophages/dendritic cells (antigen presentation and cytokine production), and T cell response (development and effector functions) [80–82]; 2) activation of Treg and