Purinergic Signalling (2013) 9:145–165
DOI 10.1007/s11302-012-9349-9

REVIEW ARTICLE

Adenosine as an endogenous immunoregulator in cancer pathogenesis: where to go?

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Received: 31 August 2012 / Accepted: 13 December 2012 / Published online: 28 December 2012

Abstract Cancer is a chronic disease and its pathogenesis is well correlated with infection and inflammation. Adenosine is a purine nucleoside, which is produced under metabolic stress like hypoxic conditions. Acute or chronic inflammatory conditions lead to the release of precursor adenine nucleotides (adenosine triphosphate (ATP), adenosien diphosphate (ADP) and adenosine monophosphate (AMP)) from cells, which are extracellularly catabolized into adenosine by extracellular ectonucleotidases, i.e., CD39 or nucleoside triphosphate dephosphorylase (NTPD) and CD73 or 5′-ectonucleotidase. It is now well-known that adenosine is secreted by cancer as well as immune cells during tumor pathogenesis under metabolic stress or hypoxia. Once adenosine is released into the extracellular environment, it exerts various immunomodulatory effects via adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) expressed on various immune cells (i.e., macrophages, myeloid-derived suppressor cells (MDSCs), natural killer (NK) cells, dendritic cells (DCs), T cells, regulatory T cell (T_{regs}), etc.), which play very important roles in the pathogenesis of cancer. This review is intended to summarize the role of inflammation and adenosine in the immunopathogenesis of tumor along with regulation of tumor-specific immune response and its modulation as an adjunct approach to tumor immunotherapy.

Keywords Adenosine · Inflammation · Tumor · Cancer · DCs · Macrophages · MDSCs · NK cells · T cells · T_{regs}

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Introduction

Cancer is a very complex disease and is characterized by plethora of changes in a large number of genes; therefore, it can be characterized as a chronic disease [1, 2]. Intense research in the field of cancer biology and its pathology has led to the establishment of six signature signs of malignant or tumor cells, which have been described very elegantly by Hanahan and Weinberg as hallmarks of cancer: (1) cancer cells keep their proliferative signaling mechanisms active and intact all the time, (2) have tendency to inhibit or evade their growth-suppressing mechanisms, (3) have power to inhibit natural cell death by apoptosis, (4) have high power of replicative immortality, (5) have tendency to induce neoangiogenesis within tumor microenvironment, and (6) have tendency to metastasize to different organs. Besides these properties, successful cancer cells coevolve with host environment and need supportive niche but the host environment also has potent destructive effects on growth and differentiation of these cancerous cells [3]. So, successful survival of cancer cells depicts the picture of failure of the host system (i.e., immune system) to control their growth and metastasis. Additionally, in later stages, supportive role of immune system for tumor cell survival and metastasis leads to advancement of cancer.

The exact cause of cancer is still unknown and more attention is now focused on the interrelationship between cancer, infection, and inflammation (Fig. 1). For example, more than 200,000 women die every year from cervical cancer, which has a close association with human papilloma virus (HPV) infection of female genital tract (i.e., cervix) [1]. *Helicobacter pylori* infection is also linked with gastrohelcosis, a form of precancerous stage of gastric cancer [4]. Studies have shown 1.9 million cases of cancer per year (2002) are mediated by different types of infections, which comprises about 17.8 % of the worldwide cancer burden [5]. For example *H. pylori* infection accounts for 5.5 % of all cancers, HPV for 5.2 %, hepatitis B and C viruses for 4.9 %, Epstein–Barr Virus (EBV) for 1 %, and HIV-1 together with human herpes virus 8 account for

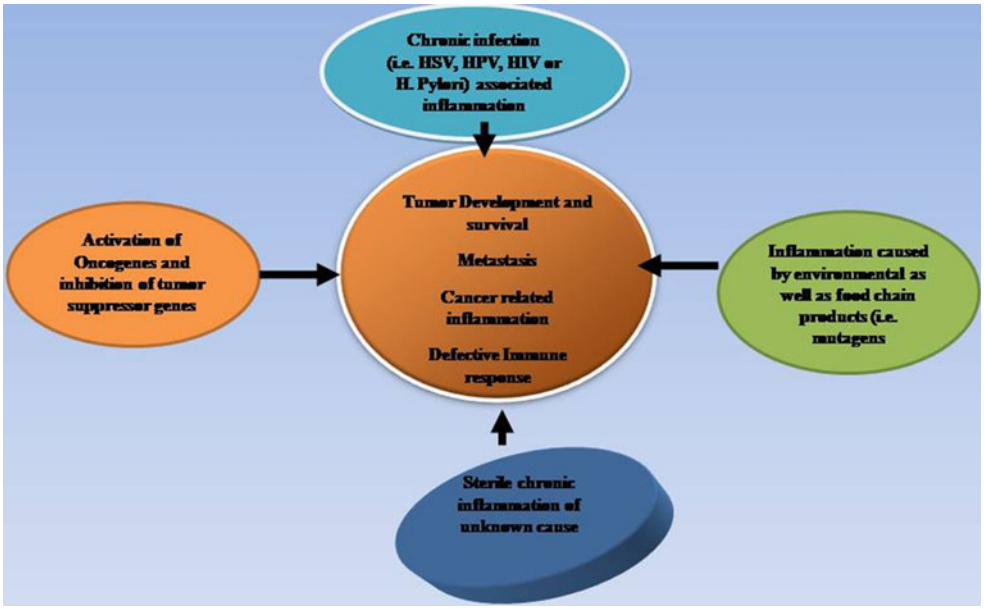

Fig. 1 Interconnection of inflammation originating from different causes and induction of cancer-related inflammation and cancer development. Accordingly, cancer and inflammation are interlinked conditions and various infections (i.e., *H. pylori*, human papiloma virus infection, hepatitis B and C virus infection, or human immunodeficiency virus infections) or sterile chronic inflammation (i.e., inflammatory bowel disease (IBD) and obesity) can lead to development to favorable conditions (i.e., induction of immune suppression) within the host for the induction and development of cancer. The cells that get transformed in this manner under these conditions start producing various supportive factors (i.e., adenosine, various cytokines, chemokines, hypoxia-inducible factor-1α (HIF-1α), cyclooxygenase-2 (COX-2), and vascular endothelial growth factor (VEGF required for angiogenesis)) that are required for the survival and differentiation of these transformed or cancerous cells. Thus, this kind of smouldering cancer-related inflammation has many tumor supportive functions

0.9 %, schistosomal infections for 0.1 %, HTLV-1 virus for 0.03 %, and liver flukes worm infections account for 0.02 % of cancers [6]. Thus, total estimated incidence was about 26.3 % of all cancers in developing countries and 7.7 % in the developed world. It will be a good therapeutic approach to fight against gastric cancer by targeting *H. pylori* infection. However, along with the infection hypothesis, the link between inflammation and cancer is also getting stronger with advancement of cancer research.

Different findings comprising of epidemiological studies of patients and molecular studies done in genetically altered laboratory animals have provided a stronger link between inflammation and cancer pathogenesis [7–10]. For example, risks of various types of cancers (i.e., bladder, cervical, gastric, intestinal, esophageal, ovarian, prostate, and thyroid cancer) increases with chronic inflammatory diseases and nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the risk of various cancers (like breast and colon cancers) [11]. Thus, chronic inflammation with (like HPV or *H. pylori* infection) or without [i.e., inflammatory bowel disease (IBD) is associated with increased risk of colon cancer] infection increases the risk of cancer (Fig. 1). This is not a new observation, but this interconnection between inflammation and cancer was made in early nineteenth century, 2,000 years ago by the Greek physician Galenus, who described the similarity between inflammation and cancer [7, 12]. According to Galenus, it may be possible that cancers may have evolved from inflammatory lesions. Thus, an important role that inflammation plays in cancer pathogenesis was known since 2,000 years ago. The Hippocratic term "cancer" was originally applied by Galenus to some types of inflammatory tumors of breast tissue, where swollen and radiated superficial veins were observed [6].

Cancer-associated inflammation is mainly characterized by the presence of inflammatory cells (i.e., macrophages, monocytes, neutrophils, etc.) as well as inflammatory mediators released by these cells (i.e., proinflammatory cytokines, various chemokines, and different prostaglandins) in tumor environment along with tissue remodeling and angiogenesis observed during chronic inflammation and tissue repair [11]. These smouldering signs of inflammation are also present in cancers for which causal linkage with inflammation is still unclear (i.e., breast cancer). However, a recent study has shown that chronic inflammation can increase the risk of recurrence of breast cancer [13]. In their study, comprising 734 women treated successfully for early stage breast cancer, higher levels of circulating acute phase proteins (APPs) approximately 3 years after treatment were found, which showed a clear association with twofold elevation in the risk of subsequent disease recurrence and mortality [13]. This data suggests that inflammation also plays an important role in breast cancer recurrence and

associated mortality. Thus, inflammation plays an important role in tumor pathogenesis and can be nominated as the seventh hallmark of cancer. However, two groups, which in true sense proposed this definition, identified a very different role of immunity and inflammation in cancer pathogenesis. For example, Colotta et al. (2009) linked inflammation to genetic instability during cancer pathogenesis [14] (Fig. 1), while Zitvogel et al. (2008) proposed defect in immunologic surveillance during cancer pathogenesis [15]. For example, besides escaping from immunologic surveillance, these tumor cells are not immunologically silent cells as tumor microenvironment is infiltrated with different immune cells [16]. These tumor-infiltrating immune cells can correlate with tumor prognosis in active progressive tumors [16, 17].

Adenosine and inflammation

Adenosine is a purine nucleoside, which plays an important role in every target organ system. Researchers involved in different fields of biomedical research are actively engaged in adenosine research due to its plethora of actions on different biologic systems (i.e., nervous, reproductive, cardiac, renal, hepatic, and respiratory systems) [18–22]. Adenosine, following its release, binds to adenosine receptors, which belong to a family of G protein coupled receptors (GPCRs) [23]. There are four different types of adenosine receptors, i.e., A_1, A_{2A}, A_{2B}, and A_3. Thus, action of adenosine is determined by the kind of a receptor to which it binds. Although adenosine is constitutively present in the biological system extracellularly at very low concentration (<1 μM), its concentration increases under metabolically stressful conditions like inflammation and cancer [24, 25]. Martin et al. (2000) have reported that plasma adenosine level rises up to 4–10 μM in patients with sepsis [24].

Drury and Szent-Gyorgyi (1929) carried out a seminal study, which demonstrated both negative ionotropic as well as coronary vasodilator properties of adenosine and, hence, the cardioprotective role of adenosine during metabolically detrimental conditions [26]. Thus, this protective effect of adenosine led to the generation of the term "retaliatory metabolite" to describe its tissue protective and remodeling action by Newby (1984) [27]. Hence, adenosine exerts its tissue-protective effect by directly decreasing energy demand of tissue as shown by its negative ionotropic effect on heart muscles and by directly increasing nutrient availability through increased vasodilation [28]; this finding can explain why tumor cells secrete adenosine in its environment.

Adenosine also plays an important role in decreasing tissue injury and maintaining their integrity is by its immunomodulatory action [28]. The immune system acts as a major player in the development of inflammation and the pathogenesis of both chronic (i.e., cancer) as well as acute inflammatory conditions. Thus, modulation of the immune system by adenosine upon its release during inflammatory insult may prove detrimental to limit the overwhelming inflammation and resulting tissue damage, but this property of adenosine has tumor-promoting consequence. Earlier findings by Jonathan et al. have shown that the extracellular concentration of adenosine in extracellular fluid of solid carcinomas may reach to 10^{-4} M (10–20-fold higher than normal concentration), which may exert a potent immunosuppressive and cancer growth-promoting effect [62]. However, according to these researchers, the concentration of adenosine was not well correlated with size of tumors.

Adenosine as a sensor for inflammation

The immune system plays a very important role in pathogenesis of both infection- as well as noninfection-associated cancer. Therefore, modulation of this diverse system by adenosine can act as a good therapeutic target to prevent inflammatory damage and, thus, mortality. However, this action of adenosine on immune system is determined by its bioavailability and adenosine receptor expression on the immune cells, which are present within tumor microenvironment. Under normal conditions, adenosine is mainly generated at the intracellular level from S-adenosylhomocysteine by S-adenosylhomocysteine hydrolase and transported across cell membranes via purine transporters [29]. Level of adenosine in extracellular environment is controlled by nucleoside or purine transporters [30, 31]. These purine transporters are divided into two main categories [32]: (1) equilibrative purine or nucleoside transporters, transporting nucleosides in/out, depending on the concentration of adenosine and (2) concentrative purine or nucleoside transporters, facilitating intracellular influx of adenosine against the concentration gradient. However, acute tissue insult or hypoxia (i.e., tumor microenvironment) causes dephosphorylation of adenosine triphosphate (ATP) into adenosine via 5′-nucleotidase enzymes (endo-5′-nucleotidase and ecto-5′-nucleotidase).

During this process, activity of adenosine kinase is also suppressed causing the inhibition of salvage activity of this enzyme and an increase in adenosine levels. For example, under hypoxic conditions during inflammation or within tumor microenvironment, inhibition of adenosine kinase causes 15–20-fold increase in both extracellular as well as intracellular levels of adenosine [231]. Generation of hypoxia-inducible factor-1 alpha (HIF-1α) during hypoxic conditions also leads to upregulation of ecto-5′-nucleotidase or CD73 activity leading to increased synthesis of adenosine [232, 233]. Besides leading to increased release of adenosine in tumor microenvironment, HIF-1α and its short isoform 1.1 also negatively regulate $CD4^+$ and $CD8^+$ T cells

leading to the decreased production of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), IL-2, IL-4, and IL-13 [234]. This study shows that HIF-1-mediated immunosuppressive action on T cells complements the tissue-protective anti-inflammatory and immunosuppressive actions of adenosine in hypoxic environment. Thus, adenosine and hypoxia both lead to immunosuppression during inflammation and tumor pathogenesis. In addition to this, HIF-1α-deficient chimeric mice develop abnormal B lymphocyte development (i.e., appearance of abnormal peritoneal B-1-like lymphocytes, with high expression of B220 (CD45) receptor-associated protein tyrosine phosphatase) and autoimmunity (i.e., anti-dsDNA antibodies and rheumatoid factor in serum, deposits of IgG and IgM in kidney and proteinuria as well as distortions of maturation of B-2 lymphocytes in bone marrow) [242]. In addition to this, T cell-specific deletion of HIF-1α in mice prevented them from acquiring sepsis and led to exaggerated proinflammatory immune response required to clear bacteria efficiently from circulation and increased their survival; these elevated HIF-1α levels may lead to immunosuppression [243]. CD73 (ecto-5′-nucleotidase) and CD39 [nucleoside triphosphate dephosphorylase (NTPD)] play major roles in maintaining high extracellular concentration of adenosine during metabolic stress (i.e., tumor) by directly catabolizing ATP into adenosine [29]. However, adenosine bioavailability is also influenced by an enzyme known as adenosine deaminase (ADA). ADA catabolizes adenosine into inosine, which further degrades into uric acid.

The inosine formed due to deamination of adenosine gets accumulated to higher concentration (>100 μM) in ischemic tissues and acts as a weak partial agonist for A_3 receptor [33]. Thus, the adenosine signaling in immune system is initiated by both upstream as well as downstream metabolites of the adenosine, i.e., adenine nucleotides exhibit a powerful immunomodulatory effect via P2-purinoceptors and inosine and uric acid also influence many facets of innate immunity [34–36]. Hence, adenosine system acts as a sensor, which provides information to the immune system about the inflammatory changes occurring in the vicinity of the immune system (Fig. 2). However, it took more than three decades to establish the role of adenosine in the pathogenesis of inflammation and the immunosuppressive properties of adenosine-mediated induction of higher levels of intracellular cAMP [235]. For example, the first two decades were spent on establishing the inhibitory role of adenosine on inflammation and showed the immunosuppressive role of elevated intracellular cAMP due to adenosine receptor-mediated signaling. This era also revealed different stimuli and conditions leading to release of adenosine into the extracellular space.

This advancement in the field of adenosine and inflammation biology interconnection led to establishment of "purinergic" receptors concept by Burnstock [236] and foundation of pharmacological approaches to modulate adenosine-mediated inflammatory processes. Now, availability of adenosine receptor gene-deficient mice advanced the field of purine biology of inflammation and inflammation-related disorders (i.e., cancer). For example, now, we can easily study the immunoregulatory role of adenosine mediated by specific adenosine receptor in vivo during acute or chronic inflammatory conditions responsible for the induction, growth, and development of tumor. This can be explained by the study performed by Ohta and Sitkovsky in 2001, where these

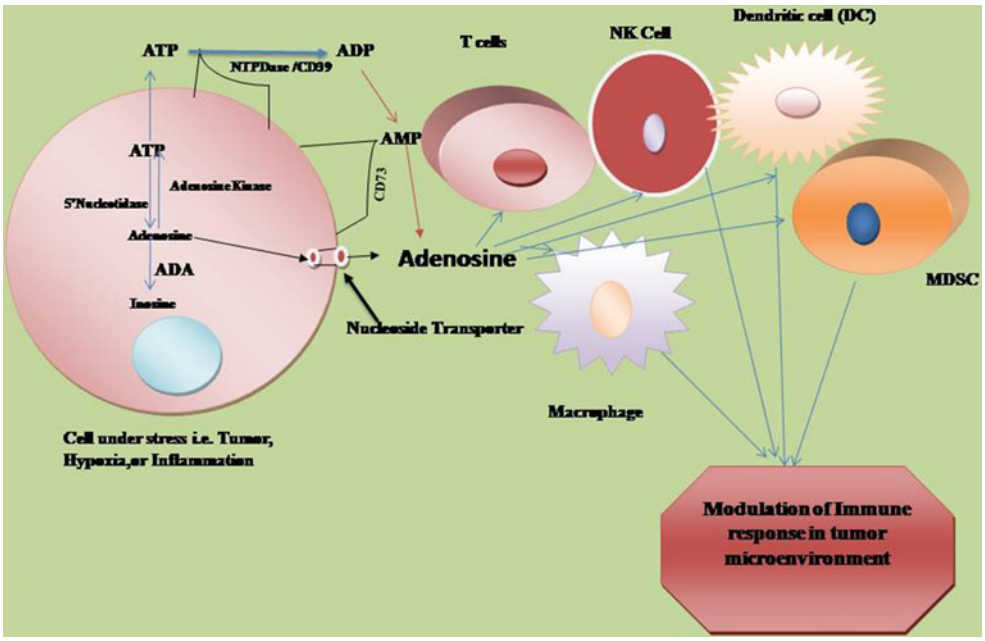

Fig. 2 Adenosine release during cancer-related inflammation and its impact on major immune cells (i.e., macrophages, NK cells, DCs, myeloid-derived suppressor cells (MDSCs), and T cells) during cancer pathogenesis

authors, for the first time, used the A_{2A} receptor knockout mice to elucidate the critical role of A_{2A} receptor activation in the down regulation of acute concavlain A (Con-A)-induced live injury [237]. In addition to this, they also confirmed the tissue-protective action of A_{2A} receptor in other models of live injury and systemic inflammation. Thus, the adenosine-mediated immunosuppressive action is mediated by its binding to A_{2AR}, causing an increase in the intracellular cAMP levels in various immune cells (i.e., macrophages, neutrophils, natural killer (NK) cells, dendritic cells (DCs), and T cells) expressing A_{2AR} in a delayed negative feedback manner [238]. Thus, during an inflammatory tissue insult, adenosine acts as a reporter, which decreases inflammatory immune signaling by binding to its sensor called A_{2AR}.

Macrophages and cancer

Macrophages have long been (almost more than 100 years ago) discovered by Elie Metchnikoff as important innate immune cells playing a vital role in phagocytosis and clearing of foreign particles (i.e., bacteria or dying cells). For example, they clear approximately 2×10^{11} red blood cells (RBCs) each day [37]. However, after their discovery till now, lots of work have been done in the field of macrophage biology and established their role in different physiological (i.e. development, homeostasis, reproduction, tissue remodeling and repair, etc.) or pathological process (i.e., fibrosis, obesity, cancer, etc.) [37, 38]. However, the role of macrophages in cancer development always encouraged researchers to identify their exact role in tumor development. This is because besides being very potent phagocytic innate immune cells they fail to clear tumor cells and promote their growth, invasion, and metastasis [39, 40].

Macrophages are categorized into different subpopulations depending on the anatomical site of their location and functional physical characteristics [41]. For example, tissue- or organ-localized macrophages include peritoneal macrophages (macrophages present in peritoneum), Kupffer cells (liver macrophages), osteoclasts (bone macrophages), histiocytes (interstitial connective tissue macrophages), pulmonary alveolar macrophages (macrophages residing in lungs), and microglial cells of the brain. In addition to these sites, macrophages are also present in eyes, testes (immune privileged sites), etc. Hence, macrophages are present widely at every compartment of the body and can be activated by both endogenous and exogenous stimuli following infection or inflammatory trauma. However, macrophages can also be stimulated by signals induced by other antigen-specific immune cells [37]. These signals produced by these specific immune cells are very clear, strong, and long lasting, which lead to alteration in macrophages for a very long period of time [42].

Classically activated macrophages (M1 macrophages)

This term is used for those macrophages that are produced during cell-mediated immune response. This response mainly involves the combination of two signals: (1) IFN-γ and (2) TNF-α signaling. Macrophages activated through both these signaling pathways have enhanced tumoricidal action and secrete higher concentration of proinflammatory cytokines and mediators [43, 44]. IFN-γ can be generated by both either innate immune cells (i.e., NK cells) or adaptive immune cells (i.e., T cells). NK cells are important innate immune cells, which play a role in tumor cell killing and tumor immunity. They respond to cells under stress (i.e., virus-infected cells) or against tumor by direct cytotoxic effect or through IFN-γ production. IFN-γ released from NK cells primes tumor-associated macrophages (TAMs) to produce further higher levels of proinflammatory cytokines and other inflammatory mediators (i.e., superoxide anions or free radicals), which have tendency to directly kill tumor cells (Fig. 3). However, production of IFN-γ by NK cells is transient and cannot sustain a population of classically activated macrophages against tumor. Besides NK cells, invariant natural killer T (iNKT) cells also produce IFN-γ and exhibit antitumor activity [45] (Fig. 3). The number of iNKT cells in peripheral blood from the patients of advanced prostate cancer was markedly decreased and their IFN-γ producing capacity was also quite low [45]. This indicates that, in patients, it may lead to impaired classical activation of macrophages helpful in removing tumor cells. This is further strengthened by a recent study in patients with gastrointestinal tumor under Imatinib Mesylate [46]. NK cell IFN-γ levels predict the long-term survival of these cancer patients [46]. Increased survival of these patients with higher IFN-γ levels upon Imatinib Mesylate treatment shows that IFN-γ exhibits its tumoricidal effect by activating the residential macrophages in tumor microenvironment and activation of direct antitumor action NK cells. Thus, therapies that can lead to generation of classically activated macrophages can become a better immunomodulatory approach to fight against cancer (Fig. 3).

However, earlier, it was believed that these M1 macrophages may contribute to the earliest stages of neoplasia [47] by releasing free radicals and promoting cell transformation. This scenario is now changed as some in vitro studies have made clear that M1 macrophages are only cytotoxic to tumor cells but not to normal cells and, therefore, these M1 cells help in the early eradication of neotrasfromed cells [48–53]. Another way by which M1 macrophages exert their antitumor activity is through their antagonistic action on tumor-promoting action of TAMs, myeloid-derived suppressor cells (MDSCs), M2 macrophages, regulatory macrophages, and immature myeloid cells (i.e., all these cells suppress adaptive tumor-specific

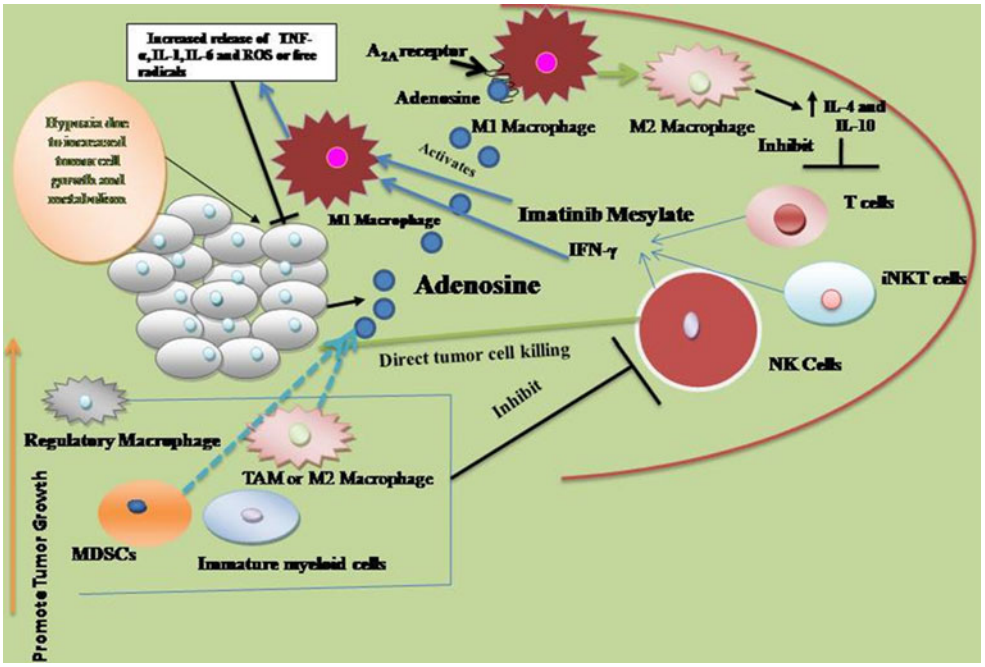

Fig. 3 Interaction of M1 and M2 macrophages in tumor microenvironment and release of adenosine by tumor cells, TAM and MDSCs leading to promote conversion of M1 macrophages to M2 phenotype. Adenosine via binding to its corresponding receptors (A_{2AR}) expressed on macrophages leads to release of IL-4 and IL-10 leading to suppression of antitumor immune response and helping the growth of tumor cells. Adenosine also leads to inhibition of release of IL-12 from macrophages leading to impairment of T cell priming and suppression of antitumor immune response. Imatinib Mesylate increases tumoricidal activity of M1 macrophages and NK cells

immune response and promote tumor growth and development) [54–59]. The exact role of macrophages in early stages of cancer is still controversial and yet to be determined. However, it generally seems to be that these cells resemble classically activated macrophages and have inflammatory as well as tumor-destructive phenotype.

Adenosine and macrophage interaction in tumor microenvironment

In tumor microenvironment, due to disordered and overwhelming growth of tumor cells or expanding cancer cells, the supportive vascular supply cannot fulfill the demand for oxygen and other required nutrient supply. This leads to development of a site that is low in oxygen and generates hypoxic stage within the tumor microenvironment. In squamous cell carcinoma of the cervix, head, and neck, the hypoxic region can be as much as 20–32 % of tumor mass [60]. As earlier mentioned, hypoxia is one of the major causes for production of adenosine; therefore, adenosine is found at higher concentration in these hypoxic sites associated with tumor. In vitro studies with 3LL Lewis lung carcinoma cells have shown that hypoxia stimulates adenosine production by tumor cells [61] (Fig. 3). In situ microdialysis studies in human and mouse models of colorectal carcinoma have also made clear that extracellular levels of adenosine were increased to more than 20-fold as compared to surrounding normal tissue environment [62]. Similar findings regarding higher extracellular levels of adenosine in solid tumor microenvironment have also been confirmed by Ohta et al. (2006) [63]. Besides being regulation of production of adenosine by CD39 and CD73 (described earlier), its levels are also controlled by dipeptidyl peptidase IV/CD26 [a binding protein for ADA] [64–67]. However, adenosine downregulates the expression of dipeptidyl peptidase IV/CD26 and its binding to ADA in colorectal carcinoma cells, which leads to further increase of extracellular adenosine levels in certain solid tumors [67].

Macrophages express all four types of adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3). Activated macrophages are capable of contributing to extracellular concentration of adenosine via generation of ATP molecules [68]. Fischer et al. (1976) have demonstrated that ADA activity increases during early monocyte differentiation into macrophages and inhibition of ADA activity decreases the process of monocyte differentiation into macrophages [69]. Najar et al. (1990) have also shown that exogenous adenosine prevented monocyte differentiation into macrophages and blocked monocyte development at a stage that resembles phenotypically to DCs [70]. Merril et al. (1997) have shown that binding of adenosine A_1 receptors expressed on monocytes promoted transformation of monocytes into multinucleated giant cells, but the binding of adenosine to A_2 receptors prevented generation of giant cells [71]. Intracellular mechanisms, which are generated by binding of adenosine to its receptors on monocytes and affecting its maturation, remain unclear and are yet to be discovered. However, the mechanism of adenosine-mediated inhibition of macrophage proliferation was discovered by Xaus et al. (1999) [72]. They showed that adenosine inhibited macrophage colony stimulating factor (M-CSF)-dependent proliferation of murine bone marrow-derived macrophages (BMDM). Adenosine induces the expression of $p^{27kip-1}$ in

a protein kinase A (PKA)-dependent pathway, causing G1 growth phase arrest of cell cycle of macrophages without inducing their apoptosis. Despite affecting the maturation of monocytes into macrophages, adenosine also suppressed the phagocytic function of macrophages by occupying the A_2 receptors [68, 73].

Macrophage recruitment to tumors is well documented in the PyMT mouse model of breast cancer [74, 75] at different stages of tumor development, i.e., adenoma/mammary intra-epithelial stage, once the tumors have progressed to an early malignancy stage [74, 76]. The same situation is also found in human endometrial and breast cancers [77, 78]. This recruitment of macrophages in this tumor microenvironment is dependent on CSF-1 (promoting the macrophage trophic phenotype) and also mediated by IL-4 and IL-10 (leading to immunosuppressive phenotype of macrophages) [79–81].

In addition to these factors, oxygen gradient may also act as a crucial factor for monocyte migration towards inflammatory as well as at the site of tumor. Local hypoxic environment impacts transendothelial monocyte migration and recruitment by upregulating the expression of endothelial cell adhesion molecules and of various chemoattracting factors, i.e., vascular endothelial growth factor (VEGF), endothelin (ET), endothelial monocytes-activating polypeptide II (EMAPII), angiopoietin-2 (Ang-2), CD11b and CD18 (α_M-integrin and β_2-integrin, respectively), and CXCL12, which contributes to fine tuning of monocyte migration into inflammatory and tumor sites [82–86]. All these conditions make M1 macrophages (tumor inhibiting) to M2 phenotype (tumor promoting) because, at this stage, there are very few hallmarks of inflammation, i.e., edema, pain, redness, and increased body temperature or fever. For example, in this scenario, it is found that macrophage polarization to tumor cell invasion-promoting phenotype is also regulated by IL-4, which is synthesized by $CD4^+$ T cells or tumor cells or both [40]. In the absence of IL-4, TAMs are incapable of promoting invasion and migration of tumor cells along with reduced metastasis in PyMT model of breast cancer [81, 87].

Adenosine receptor ligation on monocyte/macrophages and DCs inhibits the production of IL-12, which leads to impairment of T cell priming and suppression of antitumor immune response [88]. IL-12 is considered as an important antitumor cytokine, which has potent antitumor effects in mouse models of melanoma, sarcoma, kidney cancer, lung cancer, colon cancer, and ovarian cancer [89–94]. Systemic or peritumoral injection of IL-12 is capable of inducing complete regression of established tumors, limiting the formation of distant metastases, and substantially prolonging the survival of mice harboring a tumor [95]. In some tumor models, mice experiencing complete responses after IL-12 therapy were subsequently able to reject transplants of the same tumor, but not of a different tumor, which indicates that specific antitumor immunity had been established in those mice [90, 92, 93, 96]. IL-12 was found to be more effective and/or less toxic than IL-2 in models of colon, ovarian, lung, and renal cell cancer along with melanoma [90–92, 97]. Moreover, a combination of IL-2 and IL-12 was more effective than either cytokine alone in models of primary and metastatic renal cell cancer. Thus, inhibition of IL-12 cytokine release by immune cells due to adenosine prove detrimental to host suffering from cancer and provides an opportunity for tumor to grow faster and metastasize to distant organs.

All these effects of adenosine on macrophages and monocytes support the growth and proliferation of cancer cells. However, Broussas et al. (2002) found that adenosine inhibits the release of tissue factor (TF) from lipopolysaccharide (LPS)-stimulated human monocytes by binding to A3 receptors [98]. In addition to this, adenosine has also inhibited TF release from thrombin or other inflammatory mediator-stimulated endothelial cells by a nitric oxide (NO)-mediated mechanism [99]. Increase in intracellular cAMP is implicated in this inhibition of NO and TF production. This inhibitory effect of adenosine on TF release from various cells under various inflammatory stimuli leaves a question to think: is adenosine a friend or foe for host during cancer pathogenesis? This is due to the well-established fact that TF is expressed by tumor cells and contributes to a variety of pathologic processes (i.e., thrombosis, metastasis, tumor growth, and tumor angiogenesis) [100–102]. For instance, tumor cells release TF-positive procoagulant microparticles into the circulation and these may trigger venous thromboembolism in patients with cancer. TF on circulating tumor cells also coat cells with fibrin, which traps them within the microvasculature and facilitates hematogenous metastasis. Additionally, TF:FVIIa-dependent activation of PAR2 on tumor cells increases tumor growth via an undefined mechanism. Thus, all these positive effects of TF suggest that TF inhibition can prove helpful in decreasing the metastasis as well as growth and proliferation of cancer cells. Adenosine has shown this potential in different inflammatory conditions. Adenosine increases the VEGF release from macrophages, thus facilitating the process of angiogenesis [103]. Adenosine and LPS via A_{2A} receptors and toll-like receptor 4 (TLR4) synergistically upregulate the production of VEGF by macrophages in hypoxia- and NO-independent manner [104].

Hence, adenosine regulates differentiation, proliferation, and other secretory functions of macrophages by binding to the corresponding receptors they express and the analogs of adenosine can be used as future therapeutics to control inflammatory or tissue-damaging functions of macrophages in cancer.

Myeloid-derived suppressor cells in tumor environment

MDSCs are a type of immune cells, which comprise heterogenic population of immature myeloid cells including myeloid

progenitors and precursors of macrophages, granulocytes, and DCs [105]. These are mainly characterized by their suppressive effects on T cells functions [105]. In mice, these cells are characterized by the expression of CD11b and Gr-1. However, recently, they are further subdivided into two different subsets on the basis of expression of Ly-6C and Ly-6G [106]. According to this, $CD11b^{+}Ly6G^{(+)}Ly6C^{low}$ MDSCs are granulocytic in nature and are called granulocytic-MDSCs (G-MDSCs), while $CD11b^{+}Ly6G^{-}Ly6C^{high}$ MDSCs are monocytic cells and are referred as monocytic-MDSCs (M-MDSCs) (Fig. 4). It was observed that in mice bearing tumors, granulocytic MDSCs had increased concentration of reactive oxygen species (ROS) and undetectable level of NO, while monocytic MDSCs had increased level of NO but undetectable levels of ROS. These investigators found that level of MDSC-mediated T cell suppression did not depend on the expression of these molecules. This study indicates that immunosuppressive features of MDSC are caused not by expansion of a specific subset but more likely represent a functional state of these cells [106]. In cancer environment, phenotypically as well as morphologically, most MDSCs are G-MDSCs, which also express CSF-R and CD244 molecules and exhibit higher arginase, myeloperoxidase (MPO), and ROS activities [107].

However, in cancer patients, MDSCs are defined by the expression of common myeloid marker CD33, which lack markers of mature myeloid and lymphoid cells [108]. In melanoma patients, another subset of monocytic MDSC has been identified with $CD14^{+}CD11b^{+}HLA\text{-}DRI^{ow/neg}$ phenotype [109, 110]. Two main subpopulations of MDSCs taking part in suppression of immune system in melanoma and colon cancer are: $CD14^{+}$ monocytes and $CD15^{+}$ neutrophils and both of these cell types express IL-4 receptor (CD124). In patients of advanced nonsmall cell lung cancer, these MDSCs are characterized as $CD11b^{+}CD14^{-}CD15^{+}CD33^{+}$ cells [111, 112]. Thus, these findings suggest that, in human, cancer phenotype of MDSCs varies in different cancer types, but their function remains same, that is, inhibition of effective antitumor immune response mediated by T cells and help in the progression and metastasis of the tumor. Corzo et al. (2010) have shown that MDSCs in tumor-bearing mice display a biological dichotomy governed by the tumor microenvironment [113]. According to these researchers, in peripheral lymphoid organs, MDSC, by producing ROS, primarily induced antigen-specific T cell nonresponsiveness. However, within tumor microenvironment, MDSC with the same phenotype produced low ROS but dramatically higher levels of NO and exhibited higher arginase activity, leading to suppression of antigen-nonspecific T cell functions (i.e., suppression of T cell proliferation and IFN-γ production). As compared to MDSCs residing in spleen, MDSCs present in tumor microenvironment rapidly differentiated primarily into TAMs in the presence of HIF-1α. These TAMs were capable of producing

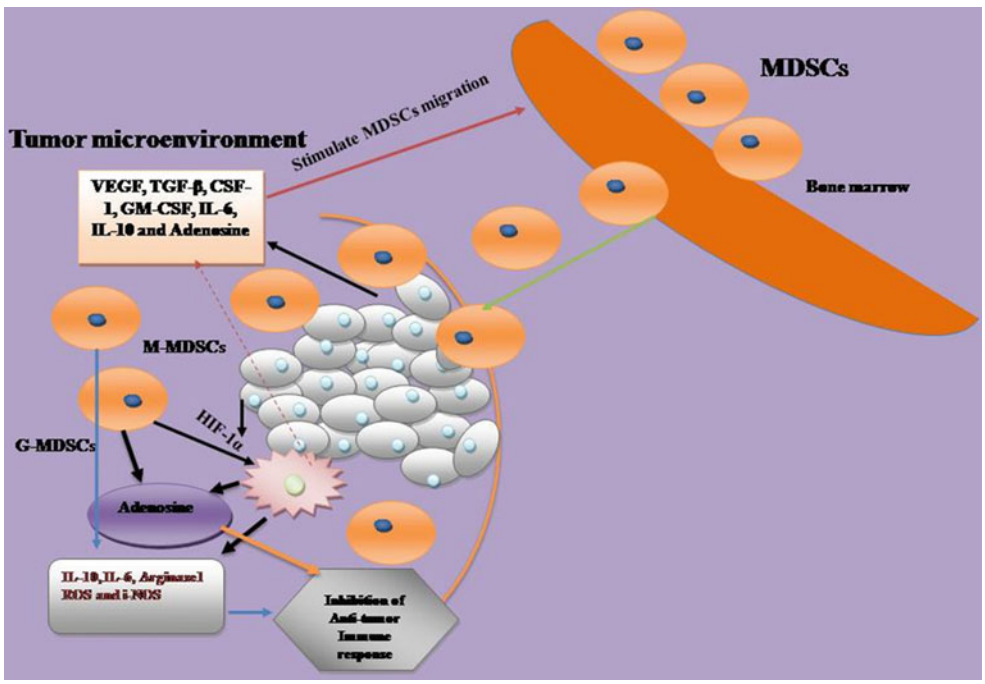

Fig. 4 Adenosine and MDSC interaction in tumor microenvironment. Due to increased energy demand, hypoxic environment in tumor mass, which leads to synthesis and release of HIF-1α from tumor cells. This hypoxia leads to generation of several tumor-derived factors (TDFs) (i.e., adenosine, VEFG, TGF-β, etc.). These factors act as major chemoattractants for MDSCs and stimulate their migration from bone marrow (BM) to tumor microenvironment. Once in tumor microenvironment, these MDSCs act as major immunosuppressor cells by releasing adenosine as well as other immunosuppressive cytokines (i.e., IL-10 and arginase-1). This extracellular increase in adenosine levels suppresses antitumor immune response mediated by DCs, NK cells, and T cells

higher levels of IL-10, arg-1, IL-6, and iNOS (Fig. 4). Thus, this study helps to explain that the difference between the nature of T cell response in the tumor microenvironment and in the peripheral lymphoid organs in tumor-bearing animals depends on MDSCs and HIF-1α interplay.

Factors responsible for MDSC infiltration and accumulation in the tumor

In healthy and tumor-free mice, MDSCs represent ~30 % of the normal bone marrow cells and <3 % of all nucleated splenocytes [113]. Their number dramatically increases in tumor environment as well as in peripheral blood and organs (i.e., spleen) in tumor-bearing mice. Earlier, it has been described that cancer is disease of chronic inflammation because cellular mediators and effector molecules of inflammation are important constituents of tumor microenvironment, and even in some types of tumors, inflammatory conditions are present before a malignant change occurs (i.e., colitis induced colon cancer or HPV induced cervical cancer, etc.). Thus, these two factors (i.e., inflammation and infection) are the two major events, which promote the migration of MDSCs into the tumor microenvironment to prevent overshooting and overwhelming immune response dangerous to host [114]. Different animal studies have provided strong evidence for the accumulation of MDSCs into the tumor site as well as in periphery (i.e., blood, spleen, bone marrow, or in lymph nodes (LN), too) and their number represents ≥20 % of all splenocytes [105, 115–118]. This accumulation of MDSCs into the tumor microenvironment is due to partial blockage of their differentiation. Recruitment of MDSCs from bone marrow to tumor microenvironment is thought to be mediated by the production of tumor-derived factors (TDFs) (i.e., VEGF, TGF-β, IL-6, IL-10, CSF-1, and GM-CSF) [119] (Fig. 4). However, all this phenomena of MDSC infiltration into the tumor site is dependent on tumor burden as well as array of TDFs produced by the tumor itself and host cells [105, 120].

MDSCs and adenosine in tumor environment

It is well characterized by early research that acute inflammation or tissue damage or tissue hypoxic condition leads to generation of adenosine intracellularly and then subsequent release of this adenosine into the extracellular environment via nucleoside (adenosine) transporters. In addition to this, adenosine can also be generated extracellularly by CD39 (NTPDase) and CD73 (Ecto5′NTase). Thus, both of these mechanisms lead to a higher level of extracellular adenosine in tumor microenvironment, which are under hypoxia. However, adenosine exerts its immunomodulatory action by binding to different adenosine receptors (i.e., A_1, A_{2A}, A_{2B}, and A_3) expressed on immune cells. A recent study by Ryzhov et al. (2011) has shown that extracellular higher levels of adenosine in tumor microenvironment are associated with the expansion of $CD11b^+Gr1^+$ MDSCs [121] (Fig. 4). This novel finding showed that adenosine is also involved in the infiltration and accumulation of MDSCs in tumor [121]. By further extending their study, they have shown that A_{2B} adenosine receptors on hematopoietic cells play an important role in accumulation of intramural $CD11b^+Gr1^{high}$ cells in mouse Lewis lung carcinoma model in vivo. These receptors enhance preferential expansion of the granulocytic $CD11b^+Gr1^{high}$ subset of MDSCs ($CD11b^+Ly6G^{(+)}Ly6C^{low}$) or G-MDSCs in vitro. Their data also showed that $CD11b^+Gr1^{high}$ subset had the highest levels of CD73 expression as compared to $CD11b^+Gr1^{-/low}$ subsets. This finding was well correlated with higher ecto-5′-nucleotidase enzymatic activity of CD73 on $CD11b^+Gr1^{high}$ MDSCs. Along with the expression of CD73, these G-MDSCs also express CD39 [121]. Thus, both enzymes required for the synthesis of extracellular adenosine are expressed on G-MDSCs (Fig. 4). This generation of adenosine by CD73 expressed on G-MDSCs may play an important role in their expansion, proliferation, and immunosuppressive activity. However, further studies are required to explore the mechanism of regulation of MDSCs by adenosine or vice versa.

Adenosine and dendritic cells interaction in tumor environment

DCs bridge innate immunity and adaptive immunity. These act as antigen-presenting cells (APCs) and have strong T cell activation potential during infection or tumor pathogenesis [122]. Immature DCs upon activation by different stimuli [i.e., pathogens or other inflammatory signals (i.e., inflammatory cytokines, alarmins, nucleotides, etc.)] lose their phagocytic activity and acquire the characteristics of mature DCs [i.e., expression of high level of major histocompatibility complex (MHC) molecules and costimulatory molecules (CD54, CD80, CD86, and CD83)] [123, 124]. During metabolic stress, in absence of TLR activation, adenosine acts as a chemotactic factor to promote the chemotaxis of immature human DCs through A_1 and A_3 receptors [125, 126]. This leads to intracellular increase of calcium concentration and actin reorganization [125, 126]. However, in fully mature DCs, adenosine strongly suppresses the TLR-induced release of IL-12 via binding to A_{2A} receptors and suppresses antitumor immune response. This inhibitory effect of adenosine on IL-12 release promotes tumor growth as IL-12 is a potent antitumor cytokine as described earlier. Adenosine increases LPS-induced expression of CD54, CD80, CD86, MHC-1, and HLA-DR molecules on

immature DCs [127]. Adenosine increases the macropinocytosis of immature DCs and increases profound release of IL-10, whereas it decreases capacity of immature DCs to activate naive T cells ($CD45RA^+$) and allogenic $CD4^+$ T cells. Both these functions of adenosine decrease antitumor action of DCs by suppressing the effective T cell-mediated antitumor immune response. Adenosine causes hypersecretion of chemokine ligand 17 (CCL17) and inhibits chemokine ligand 10 (CXCL10) release from mature DCs. CXCL10 chemokine is considered as an antitumor cytokine and has antimalignancy action [128]. Thus, decrease in CXCL10 secretion from DCs via adenosine may increase tumor growth. On the other hand, CCL17 has been linked to accumulation of $FOXP3^+$ regulatory T cells (T_{regs}) in gastric cancer [129]. In a recent study, selective knock down of CCL22 and CCL17 expression by siRNA decreased the ratios of $CD4^+$ to $CD8^+$ as well as the frequency of T_{regs} recruited by monocyte-derived DCs (MoDCs) [130]. Intratumoral injection of CCL22 and CCL17 knockout DCs significantly reduced infiltration of T_{regs} in tumor. However, an increase in the number of cytotoxic T cells ($CD8^+$T) cells in human tumor xenografts in athymic nude mice was also observed. Thus, this study indicates inhibiting the CCL17 levels within the tumor microenvironment can provide insight into cellular interactions in tumor immunology and may provide new strategy for DC vaccine development to improve cancer immunotherapy. Thus, adenosine inhibits the T cell-promoting activity of DCs through which they promote the differentiation of T cells into Th1 helper cells. Therefore, in brief, adenosine affects various activities of DCs, i.e., antigen capture regulation, expression of costimulatory molecules, cytokine, or chemokine release and also the ability of DCs to initiate and differentiate Th1 immune response. Ryzhov et al. (2008) showed that at hypoxic site (i.e., cancer and inflamed or damaged tissues), activation of adenosine receptors skews the differentiation of DCs towards a totally different cell population [131]. This change is characterized by expression of different DC and monocytes/macrophage cell surface markers and is mediated via A_{2B} receptors. In comparison to myeloid DCs, adenosine-differentiated DCs exhibit different allostimulatory activities and produce higher levels of proinflammatory, angiogenic, and immune suppressor/tolerogenic effector molecules (i.e., VEGF, IL-8, IL-6, IL-10, COX-2, TGF-β, and IDO). DCs differentiated under the influence of adenosine promote tumor growth if these DCs are injected into Lewis lung carcinoma tumors implanted in mice [132]. Thus, adenosine action via A_{2B} receptors on DCs plays an aberrant role during DC differentiation and maturation and causing a direct effect on their angiogenic, proinflammatory, and tolerogenic properties. Thus, adenosine has immunomodulatory properties and can modulate various antitumor functions of DCs during cancer pathogenesis.

Adenosine and natural killer cell interaction during cancer

NK cells are a type of innate immune cells and share common progenitor cells with T cells so they are also referred as innate immune lymphocytes. While progenitor T cells move to thymus for acquiring proper T cell characteristics, the NK cells develop extrathymically in bone marrow [133]. NK cells play a very important role in the pathogenesis of different types infectious diseases originating from different causal organisms (i.e., viral, bacterial, parasitic as well as fungal) [134–136]. They produce various cytokines and chemokines in response to these pathogens, i.e., IFN-γ, TNF-α, granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage inflammation protein (MIP)-1α, MIP-1β, and regulated on activation, normal T cells expressed and secreted (RANTES). NK cells in blood comprise 15 % of the peripheral blood lymphocytes, are also found in the liver and spleen, and in human pregnancy, they comprise the major innate immune cells (>40 %) that are present in decidua, called uterine or decidual NK cells [137–139]. NK cells form the first line of defense against various viral infections and tumors and play a major role in graft versus host disease during organ transplantation [140]. Thus, modulation of these innate immune cells may play a very important role in human health and disease.

In addition to modulation of the function of other innate immune cells, adenosine has been shown to inhibit TNF-α release from IL-2 stimulated NK cells [141]. Williams et al. have shown adenosine at concentrations ranging from 5 to 25 μM significantly inhibit granule exocytosis of NK cell stimulated with phorbol myristate acetate (PMA) by interacting with novel extracellular receptors without involving A_1 A_2, or A_3 receptors [145]. Arie et al. (2003) have shown that adenosine via binding to A_3 adenosine receptor increases antitumor activity of NK cells [142]. Raskovolova et al. (2005) have shown that adenosine and 2-chloroadenosine (CADO) inhibit the cytotoxic activity of IL-2-activated NK cells against 3LL Lewis lung carcinoma cells [61]. Thus, increased levels of adenosine in tumor environment inhibit the lytic activity of NK cells via binding to A_{2A} receptors.

Adenosine, CADO, and A_{2A} receptor agonists CGS, and 5-*N*-ethylcarboxamide adenosine (CGS21680 and NECA) inhibit both the perforin- and FasL-mediated lysis of tumor cells by lymphokine-activated killer (LAK) cells [61]. Various similar studies, in which LAK cells were isolated from deficient A_1 and A_3 receptors have confirmed that these adenosine receptors do not play any role in the adenosine-mediated suppression of NK cell activity in tumor environment [143]. However, cAMP-mediated induction of PKA via binding of adenosine to A_{2A} receptor inhibits the generation of proinflammatory cytokines from LAK cells along with inhibition of their cytotoxic action against tumor cells

in mice [144]. Adenosine also induces defective granular exocytosis in mouse NK cells via binding to an unidentified adenosine receptor and suppresses their antitumor function [145]. However, oral administration of A3 adenosine receptor agonist, i.e., 2-chloro-*N6*-(3-iodobenzyl)-adenosine-5-*N*-methyl-uronamide (Cl-IBMECA) in mice increased NK cell cytotoxicity against tumor (B16-F10 melanoma cells) and serum concentration of IL-12 [146]. Thus, by increasing the NK cell cytotoxicity and IL-12 serum level from stimulated NK cells, this A_3 agonist decreased the growth and proliferation of B16-F10 melanoma cells [146]. Hoskin et al. have also shown that 2-CADO inhibits the MHC-unrestricted cytolytic activity of anti-CD3-activated killer cells, thus suppressing the antitumor immune response [242]. Therefore, adenosine modulates the protective effect of innate immune cells (i.e., macrophages, DCs, and NK cells) towards their cancer-promoting phenotype leading to survival, growth, and development of tumor mass. This change in innate immune cell phenotype depends on the binding of adenosine to its cognate receptors on these cells or by modulating the concentration of cytokines, which affect the activity of these cells. Despite affecting the innate immune cells, adenosine also affects the adaptive immune system by modulating the function of T cells during tumor growth and development.

Adenosine and T cells

T cells comprise the major constituent of mammalian adaptive immunity and help the immune system to fight against infections (i.e., bacterial, viral, fungal, and parasitic) as well as various cancers. Thus, modulation of T cell function may play a very important role in the pathogenesis of these infectious or other noninfectious inflammatory situations (i.e., cancer). A study involving the effect of adenosine level on the function of T cells was initiated after the discovery of ADA deficiency-mediated severe combined immunodeficiency (SCID), as ADA converts adenosine into inosine and deoxyadenosine into deoxyinosine [147]. Huang et al. have shown that, at lower levels, adenosine exerted a strong inhibitory effect on the T cell receptor (TCR)-triggered proliferation and of upregulation of interleukin-2 receptor alpha chain (CD25) molecules on T cells via A_{2A} adenosine receptors without any direct lymphotoxicity [240]. T cells exhibit greater activity of ADA as compared to B cells and RBCs [148]. Higher concentration of adenosine can lead to activation of adenosine receptors expressed on T cells and may decrease their activity [149]. Both cytotoxic ($CD8^+$) and helper ($CD4^+$) T cells express A_{2A}, A_{2B} and A_3 receptors, while A_1 receptors are absent or are expressed in very low number [150, 151]. However, the $CD8^+$ T cells (i.e., antitumor $CD8^+$ T cells and human T cells) mainly express A_{2A} and A_{2B} adenosine receptors and A3 receptors are absent on these activated $CD8^+$ T cells [152, 153]. However, studies have shown that mice T cells do not have spare reservoir for A_{2A} adenosine receptors so transcriptional and translational control of A_{2A} adenosine receptors on T cells may act as an important determinants of T cell responsiveness to adenosine [241].

Binding of adenosine to these A_{2A} and A_{2B} adenosine receptors leads to increase in intracellular cAMP levels, which, in turn, leads to inhibition of TCR-mediated induction of T cell activity [154–156] (Fig. 5). Thus, binding of adenosine to these receptors expressed on T cells causes suppression of T cell-mediated adaptive immune responses like release of various immunoregulatory cytokines (i.e., IL-2, TNF-α, and IFN-γ) without significantly affecting their expansion [157, 158, 240]. However, this impairment in effector T cell function remained maintained in these T cells even after the removal of A_{2A} adenosine receptor agonist, reflecting the generation T cell memory of the immunomodulatory action of adenosine as described earlier [239]. Adenosine-mediated inhibition of IL-2 in tumor microenvironment prevents clonal expansion of immunologically active antitumor T cells. Researchers have shown that binding of adenosine to A_{2A} or A_{2B} activates protein tyrosine phosphatase SHP2, which dephosphorylates IL-2 receptor-associated STAT5 and impairs signal transduction through high affinity IL-2 receptors expressed on T cells [159]. Naganuma et al. (2006) have shown both $CD45RB^{high}$ T cells and T_{reg} cells from A_{2A} adenosine $receptor^{-/-}$ mice were aberrant, thus showing the evidence that A_{2A} receptor can differentially control expression of both pro- and anti-inflammatory cytokines released from T cells, which affects the behavior of these T cells in CD45RB transfer model [160]. Also, binding of adenosine to A_{2A} receptors on T cells modulates release of IL-12, expression of CD25 and CD69, granular exocytosis, increased regulation of Fas ligand expression, and the proliferation of T cells [161, 162]. Along with this immunosuppressive activity, adenosine has the ability to inhibit very early steps of T cell activation associated with TCR and CD28 costimulatory signaling pathways required for effective antitumor immune response against cancer [143].

Primed uncommitted $CD4^+$ T precursor Th (Thpp) cells and $FoxP3^+CD25^+CD4^+$ T_{regs}, produce immunosuppressive extracellular adenosine as both these cell types express CD73 (5′-ectonucleotidase), which has the potential to convert 5′adenosine monophosphate (5′-AMP) to adenosine [163]. Adenosine binding to $A_{2A}R$ expressed by T cells suppresses their immunostimulatory activity. Kobie et al. (2006) have shown that these $FoxP3^+CD25^+CD4^+$ T_{regs} express an extracellular 5′ectonucleotidase (CD73), which converts extracellular 5′-AMP to adenosine and suppresses T cell activity via binding to A_{2A} receptors on T cells [163].

Sitkovsky et al. (2008) have shown that FoxP3$^+$CD25$^+$CD4$^+$ T_{regs} also express CD39 ecto-ATPase/ADPase and CD73 ecto-5′nucleotidase, which shows involvement of these T_{regs} in synthesis of extracellular adenosine [164]. Lukashev et al. (2007) have observed presence of healthy normal tissue in acutely inflamed and hypoxic tissue attacked by the immune system and this protection was mediated by the release of adenosine in that vicinity, which, by binding to A_{2A} receptors expressed on T cells and other inflammatory cells, suppressed their inflammatory activity [165]. Experimental studies involving various transplantable tumors have shown that genetic deletion of A_{2A} adenosine receptors led to rejection of well-established tumors by endogenously developed CD8$^+$ antitumor T cells in approximately 60 % of A_{2A} receptor-deficient mice, whereas no rejection was observed in control wild type mice using the same number of transplanted cells [63].

Sitkovsky et al. (2008) have also shown that mice treated with A_{2A} receptor antagonists exhibited improved CD8$^+$ T cell-mediated inhibition of tumor growth, metastasis, and neoangiogenesis in cancerous tissue [164]. Thus, adenosine via binding to its corresponding receptors on T cells modulates their function, which can attribute to development of cancer and an immunosuppressive and cancer-promoting environment in the host (Fig. 3). For example, a recent study by Clayton et al. (2011) has shown that small vesicles secreted by cancer cells, known as exosomes, contribute to the increased levels of extracellular adenosine within the tumor environment [166]. They have shown that exosomes from various types of cancer cells (i.e., bladder cancer, colorectal cancer, prostate cancer, mesothelioma, breast cancer, etc.) have higher capability of phosphohydrolytic activity against ATP and 5′AMP due to expression of CD73 and CD39. Thus, exosomes secreted by cancer cells have higher tendency to increase the extracellular level of adenosine, which suppresses antitumor immune response mediated by T cells via A_{2A} receptor [166]. Inhibition of antitumor immune response mediated by T cells through their A_{2AR} in adenosine-rich tumor microenvironment may explain the paradoxical coexistence of tumors and antitumor immune cells in some cancer patients, which is called the “Hellstrom paradox” in tumor immunology. Thus, potent immunosuppressive activity of adenosine against T cells contributes to growth and development of tumor mass and tumor have evolved different strategies to generate adenosine due to its cancer-promoting and immunosuppressive action against both innate as well adaptive immune response.

Regulatory T cells in tumor microenvironment

T_{regs} are one of the highly potent inhibitors of antitumor immune response and the greatest barrier to successful immunotherapy [167]. T_{regs} are concentrated in the tumor mass during earlier stages of tumor development, leading to immune escape of the tumor from robust antitumor immune response. Thus, primary tumor can progress due to local inhibition of effector immune responses, but metastatic cells are eliminated by active systemic immune response [168]. CD4$^+$T cells, which highly express CD25 and play an important role in the suppression and prevention of autoimmunity, are referred as T_{regs} [169]. Advancement in immunology led to more specific characterization of these cells and now, these are classified as (1) naturally occurring CD4$^+$CD25^{high} T_{reg} cells, (2) induced T_{reg} cells (i.e., Tr1 and TH3 cells), and (3) CD4$^+$CD25^{high} T_{reg} cells developing in the periphery by conversion of CD4+CD25− T cells. However, all these T_{reg} cell populations coexist and play a vital role in immunosuppression [170–173]. In addition to this, induced CD4$^+$FoxP3$^+$ T_{regs} (Tr1 cells) also play an important role in immune tolerance [174]. Along with CD25, expression of FOXP3 has now been taken as uniquely expressed T_{reg} marker in mouse [175–177] and its expression is now considered as a lineage marker for developing T_{reg} cells [178, 179]. However, in humans, relying on only FOXP3 expression as a marker of T_{reg} cells should not be taken for granted because FOXP3 is also found to be expressed in activated conventional T cells without any suppressive activity [180–182].

These CD4$^+$CD25^{high}FOXP3$^+$ T_{reg} cells show a characteristic anergic state and have the tendency to suppress the CD4$^+$ and CD8$^+$T cells immune response along with DC-, NK cell-, NKT cell- as well as B cell-mediated immune response in a concentration and cell-to-cell contact-dependent manner [183–188]. In addition to the above-mentioned markers, cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and glucocorticoid-induced tumor necrosis factor receptor (TNFR)-related (GITR) protein are the most prominent molecules expressed by T_{reg} cells [189–192].

Development of T_{reg} cells is well characterized in a mouse (C57BL/6N) model of fibrosarcoma as well as in a colon adenocarcinoma model in BALB/c mice during tumor progression [193]. Transfer of unfractionated tumor-draining LN isolated on the ninth day after tumor challenge completely rejected the established tumors, whereas even fourfold higher numbers of cells harvested on day 12 provided lesser chance to prevent lethal tumor progression [193]. This was because of cotransfer of tumor-induced T_{reg} cells, which indicate that T_{reg} cells are generated or infiltrated into tumor microenvironment in a very short time span of tumor development [194]. Thus, this early induction of T_{reg} cells during tumor development may significantly affect the disease progression in human as the time point of T_{reg} cell induction in cancer patients would certainly precede the time of diagnosis in the majority of patients.

Infiltration of T_{reg} cells in tumor microenvironment

$CD4^+T_{reg}$ cells comprise of about 5–6 % of total $CD4^+$T cells [195]. In addition to naturally occurring $CD4^+CD25^+T_{reg}$ cells, other $CD4^+$ T_{reg} cells include Tr-1 cells (secrete IFN-γ and IL-10) and Th3 cells secreting higher levels of TGF-β, IL-4, and IL-10 [196–198]. Selectively larger accumulation of T_{reg} cells in tumor microenvironment was discovered in a murine fibrosarcoma model, which showed that major cell population of tumor-infiltrating lymphocytes (TILs) at later stages of tumor development and progression was comprised of T_{reg} cells. Their depletion during the effector phase of tumor progression dramatically enhanced antitumor immunity as compared to their inhibition at tumor priming stage [193].

Blockade of TGF-β and IL-10 in tumor environment led to partial removal of immunosuppression caused by T_{reg} cells, as both the cytokines are also secreted by T_{reg} cells. Additionally, TGF-β also encourages growth and development of T_{reg} cells [193, 199, 200]. Genetically engineered mice expressing a dominant negative form of the TGF-β receptor II on lymphocytes showed decreased growth of transplanted tumor [201, 202]. This effect was due to insensitivity of T_{reg} cells towards TGF-β and defect in peripheral T_{reg} cell generation [201, 202]. Similarly, it was observed when TGF-β-silenced tumor cells were engrafted into the mice [203].

However, local depletion of $CD4^+T_{reg}$ cells in tumor environment completely removed the well-established tumors and helped in the development of long-lasting immunologic antitumor memory [204]. This study suggested that T_{reg} cells suppress antitumor immune response mainly at the site of tumor but not distantly; thus, inhibition of this local immunosuppressive effect of T_{reg} cells, even at later stages of the disease, would be an effective therapeutic target against cancer [204]. This finding is further confirmed by Linehan et al. (2005) in a mouse model of pancreatic tumor, which showed that the tumors actively encourage the exaggerated accumulation of T_{reg} cells in their microenvironment by using various strategies (i.e., activation of naturally occurring T_{reg} cells as well as conversion of non-T_{reg} cells into T_{reg} cells) [205].

Immunological findings in the tumor-draining LN have made clear that the immunologic priming of both antitumor T cells and tumor-promoting $FOXP3^+$ T_{reg} cells occur in the same LN during tumor progression [206]. These authors also found that tumor antigen-specific T_{reg} cells exhibited similar functional characteristics as compared to T_{reg} cells originating from naive thymus naturally. However, it remains the hot topic of debate whether there occurs a systemic increase in T_{reg} cells in the murine model of cancer. Various findings have shown that increase in T_{reg} cells in tumor environment is not unlimited and their number never exceeds 50 % of the $CD4^+$ population [207, 208]. Once T_{reg} cells are infiltrated into the tumor microenvironment, their upper limit remains constant during the late phase of tumor progression [199]. This phenomenon of T_{reg} cell homeostasis in tumor microenvironment is well observed in gastric, colorectal, and ovarian carcinomas, where the most prominent increase in T_{reg} cells occurs during the early stages of tumor progression and remains almost unchanged at later stages [209, 210]. However, alteration of T_{reg} cell population in peripheral blood of head and neck cancer patients does not normalize in the absence of evident disease after therapy [211], which indicates that postoperative immunosuppression may contribute to relapse of the disease and even tumor metastasis.

It is well established that tumors get infiltrated with large population of T_{reg} cells, where these cells exhibit their immunosuppressive and cancer-promoting effect. Studies from a lung cancer model have shown that prostaglandin E2 (PGE2), secreted from tumor cells, acts as a chemoattractant for T_{reg} cells and resulted in their increased activity and increased FOXP3 expression [212, 213]. This was confirmed in vivo, where treatment with cyclooxygenase-2 (COX-2) inhibitor resulted in decreased activity and frequency of T_{reg} cells and lowered FOXP3 expression and tumor burden [214]. However, reverse findings were observed when mice receiving COX-2 inhibitors were treated with T_{reg} cells or given PGE2, which shows that COX-2 inhibition inhibited the T_{reg} cell-mediated cancer-promoting and immunosuppressive activity [214].

Adenosine and T_{reg} cell interaction tumor microenvironment

As described earlier, tumor microenvironment has hypoxic condition within it and extracellular adenosine-rich microenvironment, both favoring induction of development T_{regs} [215]. Additionally, increased infiltration of tumors with T_{regs} is well correlated with the poor prognosis of various kinds of tumors (i.e., ovarian cancers, nonsmall cell lung cancer, Hodgkin's lymphoma, etc.) [216–218]. Along with this, cerebrospinal fluids (CSF) of patients suffering from lymphomatous/carcinmatous neoplastic meningitis have higher number of T_{regs} as compared to normal controls [219]. There may be various unknown factors involved in the migration of T_{regs} into the tumor microenvironment, but some findings have shown that tumor-induced expression of addressins on the surface of endothelial cells selectively lead to migration of T_{regs} into the human pancreatic cancer [220]. Pancreatic adenocarcinoma cells produce CCR5 ligand and tumor-residing T_{regs} express a higher number of CCR5 than in normal tissue. This inhibition of CCR5-CCL5 signaling in mice led to decreased infiltration of T_{regs} into the tumor, indicating the potential role of CCR5 in T_{regs} infiltration into

the tumor microenvironment [221]. Besides that, CCR4-expressing T_{regs} have also been found to be highly infiltrated in the LNs of patients suffering from Hodgkin's lymphoma. Inhibition of CCR4 by a chimeric anti-CCR4 monoclonal antibody depleted $CCR4^+$ T cells and inhibited T_{regs} migration in vitro [218].

Earlier studies have shown that T_{regs} express both CD39 and CD73, which are required for the synthesis of adenosine from ATP [222, 223] (Fig. 5). Thus, T_{regs} are capable of synthesizing the adenosine molecule by its own from ATP. T_{regs} isolated from *cd39*$^{-/-}$ mice have decreased efficiency of suppressing T_{effs} from A_{2A}AR-deficient mice [223]. Thus, once the T_{regs} are infiltrated into the tumor, the synergistic effect of adenosine already present in the tumor along with the adenosine secreted by T_{regs} cause profound immunosuppression by inhibiting the release of IL-12, TNF-α from stimulated innate immune cells (i.e., macrophages and DCs) while leading the exaggerated production of immunosuppressive cytokines (i.e., IL-10). Adenosine via A_{2B} adenosine receptors impairs antitumor action of DCs [224]. While, adenosine via A_{2A} adenosine receptors directly inhibits antitumor immune response mediated by helper ($CD4^+$T cells) and cytotoxic ($CD8^+$T) cells [225]. Thus, production of adenosine by T_{regs} along with their responsiveness to this metabokine (adenosine) proves detrimental to host suffering from tumor in terms of poor prognosis of tumor. This immunosuppression is mediated by intracellular rise of cAMP levels in both T_{regs} as well as T_{effs}, as T_{regs} are capable of transferring cAMP to T_{effs} via gap junctions [226]. Both these conditions are responsible for the development of more immunosuppressive environment within the tumor microenvironment. Thus, adenosine plays an important role in the migration and regulation of immunosuppressive T_{regs} into the tumor microenvironment as well as immunosuppressive function of T_{regs}.

Future perspective

Since the very first historical finding between correlation of infections and inflammation with pathogenesis of cancer, much progress has been made in this field. This progress has led to identification of various novel pathways and molecules playing a role in the pathogenesis of cancer as the genetic deletion of these molecules have either delayed the progression of cancer or helped in the complete removal of the established tumor. Adenosine is a kind of molecule that exerts both nonimmunological (i.e., metabolic) as well immunological effects during inflammation as well as during infection-mediated inflammatory changes. Thus, adenosine plays a significant role during both infection-induced cancer development and sterile chronic inflammation leading to cancer induction. It is now well characterized that adenosine is released by tumor cells, which are under metabolic stress (i.e., hypoxia). These adenosine-mediated effects during growth and development of a tumor are induced via different adenosine receptors (i.e., A_1, A_{2A}, A_{2B}, and A_3 receptors). All these adenosine receptors are expressed by cells of the immune system and can be targeted for modulating immune response in a tumor microenvironment. Till now, various studies have been done, where either adenosine receptor agonists or antagonists have been used as novel antitumor therapeutic approach. Targeting of A_{2A} or A_{2B} receptors or inhibiting the adenosine signaling

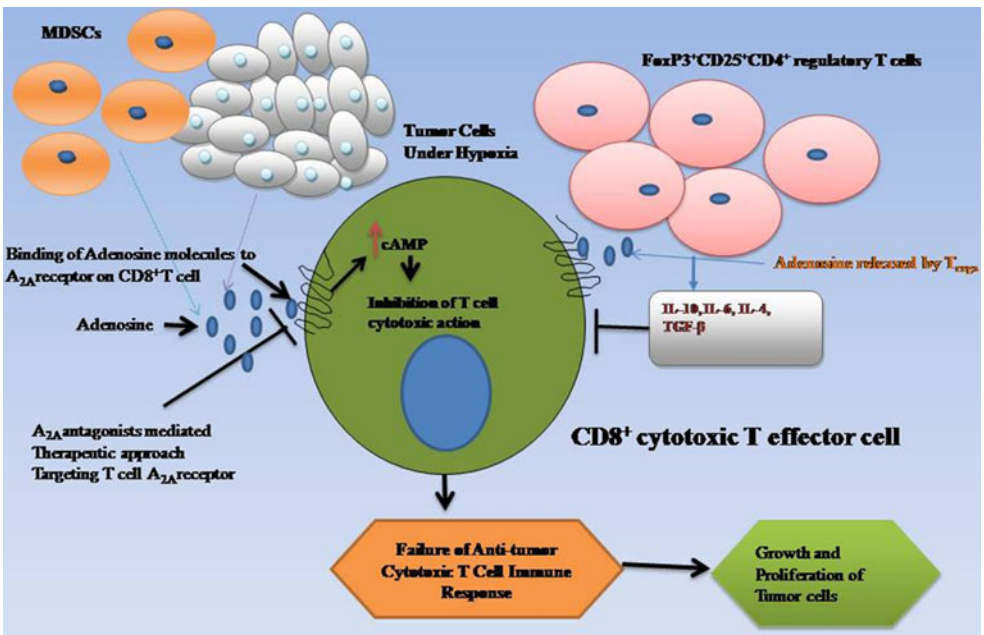

Fig. 5 Immunosuppressive effect of T_{regs}, MDSCs via adenosine by binding through A_{2AR} receptors expressed on $CD8^+$ cytotoxic T cells in tumor environment

during cancer can prove as an adjunct to tumor immunotherapy as various immunotherapeutic approaches to cancer failed either due to overexpression of CD73 on cancer cells or incredibly higher levels of adenosine within the tumor microenvironment [91]. Earlier studies have shown that genetic deletion of immunosuppressive A_{2A} or A_{2B} adenosine receptors or their pharmacological inhibition was able to prevent the inhibition of T cell-mediated antitumor immune response by the hypoxic tumor microenvironment, leading to full tumor rejection [63]. Adenosine A_{2A}, A_{2B}, and A_3 receptors play an important role in the adenosine-mediated immunosuppression [227]. For instance, studies with adenosine receptor knockout mice have shown that both A_{2A} and A_3 adenosine receptors regulate macrophage TNF-α production. As adenosine receptors are expressed by immune cells as well as the cells of every organ system, selective activation or blockade of adenosine receptors on immune cells can be used as a rational approach to treat acute or chronic inflammatory conditions (i.e., cancer). Blockade of adenosine A_{2A} receptor leads to inhibition of adenosinergic effects and inhibits immunosuppressive potential of T_{regs} in tumor microenvironment [228]. Recently, it has been shown that adenosine receptor A_3 (A_{3AR}) agonists have a protective action in various types of cancers (i.e., melanoma, prostate cancer, colon cancer, breast cancer, and hepatocellular carcinoma) through modulation of NF-κB and Wnt signaling pathways [229]. Adenosine A_3 receptor agonists have also inhibited the breast tumor-derived bone metastasis growth [230]. Thus, therapies targeting adenosine and its receptor system must require attention. Future research requires intense studies in adenosine system as a negative regulator of immune response in models of T cell-mediated cancer immunotherapies. Therefore, drug development strategies based on adenosine system and its receptor targeting should be based on both basic as well as applied research to understand the role of this system regulating or modulating the immune system during cancer. Thus, this immunomodulatory therapeutic approach should first ensure the fine balance between the tumor-destructive and tumor-promoting immune response, that is, it should only inhibit the tumor-promoting adenosine-mediated immunosuppressive immune response (i.e., inhibition of T_{regs}, TAMs, and MDSCs), while leaving or promoting the antitumor (i.e., cytotoxic T cell, M1 macrophage, and NK cell) immune response intact.

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