

Role of adenosine in tumor progression: focus on A_{2B} receptor as potential therapeutic target

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ABSTRACT

Adenosine receptors are a family of G-coupled receptors which mediate the anti-inflammatory and immune-suppressive effects of adenosine in a damaged tissue. A large number of evidence indicate that the accumulation of adenosine under hypoxic conditions favors tumor progression, helping cancer cells to evade immune responses. Tumor cells and/or lymphoid and myeloid cells can express the adenosine-generating enzyme CD73 and/or A_{2A} receptor, which in turn strongly suppresses an effective T-cell-mediated response, while promotes the activity of suppressive cells such as Treg and myeloid-derived suppressor cells. CD73 inhibitors and A_{2A} antagonists, either as single agents, or in combination with immune-checkpoints inhibitors such as anti PD-1 monoclonal antibodies, are currently in Phase I clinical trial in cancer patients. Recent studies show that A_{2B} receptor plays an important role in mediating the pro-tumor effects of adenosine, since its selective blockade can inhibit tumor growth in some murine tumor models. Targeting A_{2B} receptor reduces immunosuppression induced by myeloid cells and inhibits the stromal cells activity within the tumor microenvironment, limiting tumor angiogenesis and metastatic processes. Here, the authors review the current data on involvement of A_{2B} receptor in regulating tumor progression and discuss the development of A_{2B} receptor inhibitors as potential therapeutic agents in cancer treatment.

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INTRODUCTION

Tumor microenvironment is populated not only by malignant cells but also by other stromal cells and immune cells that cooperate to the development of cancer.^[1,2]

In the eternal battle against cancer, several strategies have been developed. One of the first approach to treat cancer has been the antineoplastic chemotherapy which is made up of chemical substances that provide to halt directly the highly-replicating tumor cells by damaging their RNA or DNA.^[3] Radiotherapy is another important



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treatment currently used for several tumors, through which cancer cells are either directly killed upon DNA damage by depositing high physical energy of radiation, or indirectly due to the release of free radicals.^[4] Nowadays the most novel anti-cancer strategies are the targeted-therapy and immunotherapy. The cancer targeted-therapy uses small molecules that can block fundamental pathways or mutant proteins essential for tumor growth.^[5] Conversely, cancer immunotherapy is a therapeutic strategy that improves the host immune response against cancer cells, instead of acting directly on tumor cells.^[6]

Several chromosomal alterations, genetic mutations and genomic instability that occur in cancer cells provide a different set of antigens that the immune system can use to distinguish transformed cells from their own cells.^[7] However, tumor cells escape from host immune surveillance through different mechanisms, that include loss of immunogenicity and ineffective T-cell mediated responses. Moreover, several inflammatory mediators including chemokines [CC-chemokine ligand 2 (CCL2), CCL5, CXC-chemokine ligand 1 (CXCL1), CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8, CXCL10 and CXCL12], cytokines [tumor necrosis factor (TNF), interleukin 1 (IL-1), IL-4, IL-5, IL-6, IL-10 and IL-13] and growth factors [granulocyte macrophage-colony stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), transforming growth factor- β (TGF- β)] are released by tumor cells and/or stroma and immune cells surrounding tumor tissue, generating a chronic inflammatory microenvironment. Chronic inflammation in cancer can facilitate tumor proliferation and invasion and drive the recruitment and activation of immunosuppressive cells, including T regulatory (Treg) cells, myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAM). In this context, many inhibitory receptors, known as “immune checkpoint molecules” such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and programmed cell death-1 (PD-1), are upregulated on activated lymphocytes during an active immune response providing a negative feedback mechanism.^[7] CTLA4 binds to members of the B7 family on antigen-presenting cells (APCs) inhibiting T-cell activation, while PD-1 interacts with ligands PD-L1, expressed on different cell types including tumor cells, or PD-L2 on macrophages and dendritic cells, inhibiting T-cell functions.^[7,8] The development of agonist antibodies (for costimulatory pathways) or antagonist antibodies (for inhibitory pathways) which target lymphocyte receptors or their ligands is one of the most promising approach with the potential to modulate the tumor microenvironment and improve the efficacy of immune response/s against cancer

cells.^[8] The first class of immunotherapeutics approved by US Food and Drug Administration (FDA) for patients with metastatic melanoma includes antibodies against CTLA4 (Ipilimumab and Tremelimumab).^[9] Later on other immune checkpoint molecules have been discovered, such as antibodies against PD-1 (Nivolumab, Pembrolizumab and Atezolizumab), PD-L1, lymphocyte-activation gene 3 (LAG3, also known as CD223), B7-H3 (also known as CD276), B7-H4 (also known as B7-S1, B7x and VCTN1) and T-cell immunoglobulin domain and mucin domain 3 (TIM3).^[8] The therapeutic outcomes in cancer patients is improved by combining immunotherapeutics with chemotherapy.^[8] The concomitant blockade of different immune checkpoints may increase the success of immunotherapy in cancer patients.^[8] Hence, in the last few years many efforts have been made aiming to investigate novel therapeutic strategies to inhibit cancer-induced immune-suppression. It has become clear that in the tumor microenvironment there are several pathways that may play an important role in the tumor immune evasion process. Among them, extracellular adenosine, an ATP-derived molecule generated by the extracellular CD39/CD73 enzymes, has been identified as an immune checkpoint that critically impairs the anti-tumor immune response mainly via A_{2A} adenosine receptor subtype.^[10-12] Accordingly, selective inhibitors of adenosine signaling pathways have been tested in pre-clinical studies^[13,14] and some of them, including the antibody anti-CD73 and the A_{2A} receptor antagonists are currently in Phase I clinical trials in cancer patients, either as single agents, or in combination with immune checkpoints inhibitors such as anti PD-1 monoclonal antibodies [NCT02503774 and NCT02655822].

While the role in tumor immunity of CD73-A_{2A} receptor axis has been extensively examined, less is known about the role of A_{2B} receptor subtype in tumor development and progression. Compelling evidence suggest that this receptor contributes to the pro-tumor effects of adenosine within tumor microenvironment. In this article, we review the current data on the effects of adenosine in tumor progression, focusing on the emerging role of A_{2B} receptor in regulating tumor growth and discuss the therapeutic potential of targeting A_{2B} receptors in cancer treatment.

CRITICAL ROLES OF ADENOSINE IN TUMOR PROGRESSION

Adenosine is a key endogenous molecule produced at the extracellular level by two ectoenzymes, ecto-5'-nucleotidase (CD73) and ectonucleoside triphosphate diphosphohydrolase-1 (CD39) physiologically

expressed on both hematopoietic and non-hematopoietic cell types.^[15,16] Once released in the extracellular space, adenosine elicits its physiological responses by coupling and activating four membrane adenosine receptors (A₁, A_{2A}, A_{2B}, A₃) which contain seven transmembrane domains coupled to G proteins.^[17] Once bounded with its receptors, adenosine can inhibit (via A₁ and A₃) or stimulate (via A_{2A} and A_{2B}) the adenylyl cyclase resulting in a decrease or increase in cyclic AMP (cAMP) accumulation, respectively.^[18] cAMP activates the protein kinase A (PKA) and in turn the nuclear substrate cAMP responsive element-binding protein (CREB) that regulates the expression of several genes by binding to cAMP responsive elements and other cAMP effectors such as Epac, altering pro-inflammatory genes expression.^[19-22] A_{2B} receptor can also activate the phospholipase C (PLC) by coupling Gq protein.^[23,24] All adenosine receptors are involved in the modulation of mitogen-activated protein kinase (MAPK) activity.^[25]

The tumor milieu is characterized by high levels of adenosine triphosphate (ATP) due to the high proliferating rate of cancer cells. The ATP is rapidly converted at the extracellular level in ADP and AMP through two reversible steps via CD39, while the last irreversible step in adenosine is mediated by CD73.^[16] Under homeostatic conditions adenosine level is low but during pathophysiological events (including stress, infection, inflammation and cancer) extracellular adenosine levels can be increased from 10-200 nmol/L up to 10-100 μmol/L.^[26] In inflammatory-associated conditions, adenosine typically attenuates the inflammatory response.^[26,27] Importantly, studies by Ohta and Sitkovsky^[28] showed for the first time that A_{2A} receptor-deficient mice are unable to control inflammation, resulting in exaggerated immune responses which can trigger extensive tissue disruption with subsequent cell death. These effects of adenosine are dependent on the activation of the adenosine A_{2A} receptors on immune cells, which induce a wide range of singular immunosuppressive responses which regulate the uncontrolled inflammation to harmful insults.^[27,29,30] However, in the context of tumor while extracellular ATP increases the T-cell mediated effector function, high levels of adenosine mediates opposite effects favoring immune suppression that is associated with tumor growth and metastasis.^[31] Hypoxia, which is a common feature of the tumor microenvironment that promotes immunosuppression, is one of the main factors responsible of the increased production of adenosine within many solid tumors.^[11] Indeed, the expression and the enzymatic activities of CD39 and CD73, responsible of the adenosine generation, increased under hypoxic conditions, while the expression of the

adenosine kinase, which inhibits the metabolism of adenosine, is down-regulated.^[11] At the same time, the expression of adenosine receptors A_{2A} and A_{2B} is also up-regulated.^[32] Consequently, adenosine along with other HIF-induced immunosuppressive factors and cells, contributes to modulate the functions of tumor cells, tumor-infiltrating immune cells and/or other stroma cells.

Within the hematopoietic compartment, CD39 is expressed on B cells and monocytes, subsets of CD8⁺ T cells, CD4⁺ T cells and NK cells.^[15,33] CD73 is expressed on B cells and subsets of CD8⁺ T cells, CD4⁺ T cells and NK cells and small subsets of monocytes.^[15,33] CD39 and CD73 are co-expressed on B cells, Treg cells, Th17 cells, NK cells, neutrophils, tissue macrophages and myeloid-derived suppressor cells (MDSCs).^[15,33] CD39 and CD73 are also expressed on endothelial cells and on the surface of several types of cancer cells.^[14,15] Thus, CD73-expressing cells, including immune cells and/or stroma cells, produce adenosine that accumulate in the tumor microenvironment and profoundly impairs anti-tumor immune responses. Accordingly, a large number of evidence have proved that targeting adenosine-generating enzymes significantly reduces tumor growth by improving anti-tumor immune responses.

A_{2A} receptor is the most thoroughly characterized receptor involved in the adenosine-induced anti-inflammatory/immune-suppressive effects within the tumor microenvironment. A_{2A} receptor is highly expressed on lymphocytes, macrophages, dendritic cells, NK cells, and neutrophils. Activation of A_{2A} receptor significantly reduces T-cell receptor (TCR)-triggered effector functions, including proliferation and production of cytokines and chemokines, preventing T cells activation and function via cAMP/protein kinase cAMP-dependent (PKA) pathways.^[34-36] These effects occurs upon A_{2A} adenosine receptor stimulation in naïve CD4⁺ T cells as well as in CD8⁺ T cells. Furthermore, A_{2A} receptor stimulation reduces the expression of CD25 and CD40 ligand (CD40L) and increases the expression of PD-1 and CTLA-4 on T cells,^[37] inducing T cell anergy that promotes peripheral tolerance.^[35] Stimulation of A_{2A} receptor on myeloid cells can also affect the release of IL-12 and induce the production of IL-10,^[38] affecting significantly the T- and NK-cell responses in the solid tumor microenvironment.^[39] Additional evidence also show that A_{2A} adenosine receptor stimulation promotes the development of immune suppressive myeloid cells^[40] or Treg cells.^[41] The first *in vivo* genetic evidence of the role of A_{2A} receptor in tumor progression has been reported by Ohta *et al.*^[10] who showed that 60%

of A_{2A} receptor deficient mice completely rejected established immunogenic tumors in a CD8⁺ T-cell-dependent manner. However, 40% of tumor-bearing A_{2A} receptor deficient mice did not reject the tumor, possibly because of the expression of A_{2B} receptor on A_{2A} receptor deficient CD8⁺ T cells.^[10]

At the same time a large number of evidence show that inhibition of CD73 activity or CD73 knockdown on tumor cells inhibit tumor growth and metastasis by enhancing the anti-tumor T cell response.^[42-46] CD73-deficient mice are resistant to tumor and show an increased influx of CD8⁺ T cells^[44] and low number of Tregs within the tumor.^[47]

The expression of CD73 on various tumor cells from cancer patients, including breast,^[46] glioblastoma,^[48] prostate,^[49] ovarian,^[50] leukemia^[51] has been associated with poor prognosis. Notably, some chemotherapeutics are able to increase the expression of CD73 on cancer cells, which may in turn represent a putative mechanism of resistance to chemotherapeutics.^[46,49,51] On the other hand, targeting CD73 can improve the therapeutic potential of some conventional cancer treatments, including chemotherapy, radiotherapy and immunotherapy. For example, inhibition of CD73 in combination with doxorubicin prolonged the survival of mice with metastatic breast cancer.^[46] Adenosine can also impair the anti-tumor response induced by high dose of radiation therapy.^[52] Administration of CD73 inhibitor into mice with tumors exposed to radiation therapy can significantly reduce tumor growth.^[52] Notably, inhibition of CD73 may also improve the synergy of radiation therapy in combination with anti-CTLA4 monoclonal antibody.^[52]

Recent studies indicate that inhibition of adenosine/A₂ adenosine receptors axis synergizes with other immune checkpoints inhibitors reducing potently tumor growth in murine models of cancer. In particular, treatment of mice with monoclonal antibody anti-CD73 enhances the anti-tumor effects of antibodies anti-PD1 and anti-CTLA4.^[53] In support, other studies have demonstrated that selective blockade of A_{2A} adenosine receptor in combination with anti PD-1 antibody and anti-CTLA4 antibody potently reduced tumor growth.^[54-56] The therapeutic synergy of these combinations depends on the CD73 expression on tumor cells, proving that CD73-generating adenosine by tumor cells within the tumor microenvironment may affect the activity of immunotherapy. Furthermore, blockade of PD-1 enhances the expression of A_{2A} receptors on tumor-infiltrating CD8⁺ T cells, suggesting that adenosine via A_{2A} receptor limits the immune

response against cancer induced by inhibitors of immune checkpoints.^[54] More recently, it has been demonstrated that blockade of A_{2B} adenosine receptor subtype with a selective antagonist improves survival and the anti-metastatic effects of anti-PD1 and anti-CTLA4 monoclonal antibodies in both melanoma and mammary cancer models of metastasis with cells expressing CD73.^[57] The anti-metastatic effects of these combinations relies on the capacity of immune checkpoints inhibitors to boost immune responses and on direct effects of A_{2B} adenosine receptor inhibitor on cancer cell metastasis.^[57] Here the authors show that blockade of A_{2B} receptor in A_{2B} receptor deficient mice is able to reduce the metastasis of human triple negative breast cancer (TNBC) xenografts, confirming the critical role of A_{2B} receptor on cancer cells rather than host cells.^[57] Altogether these preclinical studies strongly support the therapeutic potential of targeting adenosine in cancer.

Experimental evidence suggests that also CD39 can represent a potential therapeutic target for cancer treatment. CD39 is highly expressed by Treg cells and together with CD73 generate adenosine in the tumor microenvironment.^[58] Elevated levels of CD39-expressing Treg cells have been found in some mouse tumor tissues, including melanoma and colorectal cancer.^[58] Inhibition of CD39 reduces the tumor growth, enhances the recruitment of T cells in the tumor lesions and improves the effector functions of CD8⁺ T cells and NK cells, by impairing the activity of CD39-expressing Treg cells.^[58] Although additional studies are needed to better clarify the therapeutic potential of targeting CD39 in cancer, the use of CD39 inhibitors might be useful to limit the immune suppression induced by Treg cells.

Selective agonists of A₃ adenosine receptor subtype have proved to directly inhibit proliferation of A₃-expressing tumor cells by arresting cell cycle progression and exert immunostimulatory effects in some murine tumor models in a NK- and T-cell-dependent manner, enhancing the production of Th1-like cytokines in the tumor microenvironment.^[59-63] A₃ adenosine receptor agonists have been tested indeed in some clinical trials for rheumatoid arthritis (NCT00280917, NCT00556894, NCT01034306, NCT02647762),^[64] hepatocellular carcinoma (NCTNCT00790218, NCT02128958) and hepatitis (NCT00790673),^[65] dry eye syndrome (NCT01235234, NCT00349466)^[66] and psoriasis (NCT01265667).^[67]

Nonetheless, emerging evidence suggest that A_{2B} receptor can mediate the pro-tumor effects of adenosine. It is known that A_{2B} receptor is important

in some patho-physiological conditions, including vascular injury,^[68] chronic lung disease,^[69] vascular leak,^[70] and ischemic disease.^[71] First studies performed by Ryzhov *et al.*^[72] in 2008 show that tumor growth in A_{2B} receptor deficient mice was reduced compared to that observed in wild type mice, providing the first genetic evidence for a pivotal role of A_{2B} receptor in tumor progression.

Up to now a number of selective A_{2B} receptor antagonists (such as MRS1754, ATL801, GS-6201, PSB603 and PSB1115) and selective A_{2B} agonists (Bay60-6583) have been synthesized, helping the study and the characterization of the role of this adenosine receptor in many patho-physiological conditions, including cancer, as discussed below.

EXPRESSION OF A_{2B} RECEPTOR

A_{2B} adenosine receptor is widely expressed in the entire organism, although its role is not completely understood. The A_{2B} receptor expression has been detected in type II alveolar epithelial cells,^[73] endothelial cells,^[74] chromaffin cells,^[75] astrocytes,^[76] neurons,^[77] and taste cells.^[78] Moreover, A_{2B} receptor is expressed also on many immune cell populations including mast cells,^[79] neutrophils,^[70] dendritic cells,^[80] macrophages,^[74] and lymphocytes.^[81]

Despite A_{2B} receptor binds adenosine with lower affinity (EC₅₀ = 24 μmol/L) than A_{2A} receptor,^[72,82] its relevance in regulating tumor growth is becoming clear both because its expression is highly influenced by the tumor milieu and because A_{2B} receptor can play different physiological roles compared to A_{2A} receptor.

The tumor microenvironment is characterized by high proliferating rate of cancer cells which contribute to hypoxia condition. Hypoxia is a very strong stimulus for up-regulating A_{2B} receptor expression through hypoxia inducible factor (HIF-1α) and hypoxia-dependent signaling pathways in endothelial cells, dendritic cells (DCs), muscles, fibroblasts and T cells.^[32,83-87] Indeed, a functional hypoxia-responsive region within the A_{2B} receptor promoter has been identified, confirming the selective transcriptional induction A_{2B} receptor by hypoxia.^[87] Transcription of A_{2B} receptor can be induced by bacterial lipopolysaccharide (LPS) or interferon (IFN)-γ in macrophages,^[88,89] by TNF-α in vascular smooth muscle cells,^[90] and by IL-1β in endothelial cells.^[91] Furthermore, a post-transcriptional regulation of A_{2B} receptor by inflammatory mediators has been demonstrated in endothelial and pulmonary epithelial cells^[92] and in colonic epithelial cells.^[93] Therefore, although A_{2B} is a low-affinity adenosine receptor,

under inflammatory-hypoxic conditions, its expression is up-regulated while the concentration of adenosine reaches highest levels. In this context, the A_{2B} receptor may play an important role in mediating adenosine-induced pathological effects.

A_{2B} RECEPTOR AND TUMOR IMMUNITY

Although the role of A_{2B} receptor in controlling T-cell-mediated response is not completely clear, compelling evidence indicate that this receptor may influence the features of some immune cell populations.

It has been demonstrated that A_{2B} receptor is involved in the differentiation of T cells under Treg skewing-conditions, since its inhibition is able to suppress the expression of FoxP3 and IL-10 production in a way completely independent from T cell activation.^[94]

To be activated and provide anti-tumor responses CD4⁺ T-cells need the expression of the major histocompatibility complex (MHC) class II. In several types of tumors, the loss of MHC class II is related to impaired levels of CD4⁺ T-cells.^[95] Moreover, the levels of either MHC class II or class II transactivator (CIITA) are altered in highly metastatic cancer cells.^[96] A_{2B} receptor stimulation by repressing CIITA can impair MHC class II transcription in IFN-γ-stimulated cells.^[97,98] Moreover, bone marrow-derived dendritic cells (BMDCs) express A_{2B} receptor and adenosine inhibits BMDCs IL-12p70 production via A_{2B} receptor. Depending on the levels of this cytokine, CD4⁺ T-cells can differentiate into Th1 or Th2 cells.^[99] The impaired production of pro-inflammatory cytokines (TNF-α and IL-12) and the increased IL-10 production induced by A_{2B} receptor activation leads to a lower expression of CD86 and MHC class II lowering CD4⁺ T cell stimulation.^[100]

A_{2B} receptor can also affect macrophages proliferation induced by macrophage colony-stimulating factor (M-CSF)^[101] and the differentiation of human monocytes, mouse peritoneal macrophages and hematopoietic progenitor cells (HPCs) into myeloid DCs with tolerogenic and angiogenic features.^[80] A_{2B} receptor activation promotes the expansion *in vitro* of MDSCs, that contribute to induce immunosuppression by producing adenosine.^[102] MDSCs potently suppress anti-tumor T-cell response and/or promote angiogenesis.^[103] Altogether, these studies strongly support a role of A_{2B} receptor in inducing the differentiation of hematopoietic progenitor cells into mature cells with tolerogenic and suppressive features. Subsequent studies performed *in vivo* show that A_{2B} deficient mice have reduced amounts of tumor-

infiltrating myeloid cells CD11b^{high}/Gr-1^{high}, suggesting that A_{2B} receptor suppresses immune surveillance.^[72] Later, Cekic *et al.*^[104] showed that the selective blockade of A_{2B} receptor inhibits bladder and breast tumor growth in mice, by inducing a T-cell mediated response in a CXCR3-dependent manner. In a mouse model of melanoma, selective blockade of A_{2B} receptor inhibits tumor growth.^[105] This effect was associated with lower levels of IL-10 and MCP-1 in the tumor tissue and reduced accumulation of tumor-infiltrating MDSCs.^[105] Notably, the levels of MDSCs in secondary lymphoid organs remained unchanged in mice treated with the selective A_{2B} receptor antagonist, consistent with a selective activity of the antagonist on the recruitment of MDSCs to tumor lesions rather than with a putative systemic effects.^[105] Blockade of A_{2B} receptor within the tumor microenvironment modulates the intra-tumoral levels of various inflammatory mediators and growth factors that could in turn influence the features of tumor-infiltrating immune cells, promoting the recruitment/accumulation of MDSC.^[106] Accordingly, the percentage of tumor-infiltrating CD8⁺ T cells upon A_{2B} receptor blockade enhanced in the tumor lesions.^[105] Furthermore, treatment of mice with the A_{2B} receptor antagonist PSB1115 in combination with dacarbazine, a chemotherapeutic agent commonly employed in melanoma patients, reduces tumor growth and significantly increases the number of CD8⁺ T cells in the melanoma lesions demonstrating the high potential of combining A_{2B} receptor blockade and chemotherapy for cancer treatment.^[105,106]

In conclusion, the experimental evidence in some tumor mouse models suggest that the selective blockade of A_{2B} receptor may ameliorate T cell-mediated immune surveillance by impairing the accumulation of suppressive cells and the levels of inflammatory factors in the tumor microenvironment.^[72,104-106] However, despite the relevance of these observations, more studies are needed to provide a detailed understanding of the role of A_{2B} receptor in modulating the immune responses in tumor environments.

A_{2B} RECEPTOR AND TUMOR STROMA

A number of studies indicate that A_{2B} receptor can directly affect the proliferation/migration of tumor cells and the function of other stroma cells that populate the tumor niche, including endothelial cells and fibroblasts.

A critical role for A_{2B} adenosine receptor in mediating proliferation and/or apoptosis in different cancer cell lines has been delineated. A_{2B} adenosine receptor is highly expressed in prostate cancer cell lines

and selective antagonist of A_{2B} adenosine receptors or silencing A_{2B} receptors blocked the proliferative effects induced by a non-selective adenosine analog NECA.^[107,108] Other studies indicate that A_{2B} adenosine receptor is highly expressed also in oral squamous carcinoma cell lines, as well as in human oral carcinoma tissues, where its expression is correlated with those of HIF-1.^[109] Studies by Gessi *et al.*^[110] demonstrate that in colon cancer cells, although at the mRNA levels A_{2B} receptor is more expressed than A₁, A_{2A} and A₃, the density of A₃ receptors is the highest among the adenosine receptor subtypes. Later, other studies have demonstrated that the adenosine A_{2B} receptor is up-regulated in colorectal carcinoma tissues and colon cancer cell lines compared with normal colorectal mucosa under hypoxic conditions.^[111] Antagonists of A_{2B} receptors inhibit cancer cell proliferation, suggesting that this receptor may be a potential therapeutic target for colorectal cancer.^[111]

In contrast, in gastric cancer cells A_{2B} adenosine receptor has been identified as target of miR-128b, a proto-oncogene miRNA down-regulated in gastric cancer tissues.^[112] In this work, the authors demonstrate that the down-regulation of miR-128b in gastric cancer cell is associated with an over-expression of A_{2B} adenosine receptor and decreased cell apoptosis rate.^[112] In osteosarcoma cells it has been demonstrated that p73 upregulates A_{2B} adenosine receptor and A_{2B} receptor agonists can enhance p73-dependent cell death in response to chemotherapy.^[113] Moreover, stimulation of A_{2B} receptor with a non-selective adenosine analog NECA induces apoptosis in ovarian cancer cells.^[114] Nonetheless, while a number of studies demonstrate that stimulation of A_{2B} adenosine receptor in some cancer cell types promotes proliferation, whereby knockdown or pharmacological inhibition of this receptor reduces tumor cell growth and promotes apoptosis,^[107-111] opposite results have been also described.^[112,113] The discrepancy might likely depend on the cancer cell types, the expression levels of this receptor on tumor cells and the selectivity and/or concentrations of pharmacological tools used in the experimental settings.

It has been demonstrated that agonists of A_{2B} receptor induce anti-proliferative and pro-apoptotic effects on glioblastoma cancer stem cells (CSCs).^[115] Furthermore, stimulation of A_{2B} receptors as well as A₁ receptors sensitize glioblastoma CSCs to chemotherapy.^[115]

A role of A_{2B} receptor in promoting the migration of tumor cells *in vitro* and *in vivo* has been clearly

demonstrated. Indeed, a number of studies show that adenosine may directly influence the migration/invasion of tumor cells via A_{2B} adenosine receptor. Stagg *et al.*^[42] have demonstrated that targeting the adenosine-generating enzyme CD73 inhibits tumor growth in mice and significantly delays the development of spontaneous lung metastasis. While the effect of anti-CD73 monoclonal antibody therapy on primary tumor growth relies on its capacity to improve immune surveillance, the anti-metastatic effects to the lungs is rather dependent on a direct effect of CD73-generating adenosine on breast tumor-cell migration via A_{2B} adenosine receptors stimulation.^[42] Consistent with the role of A_{2B} receptor in promoting metastasis of breast cancer cells to the lung, administration of selective or non-selective A_{2B} receptor antagonists into mice significantly reduced metastasis burden.^[42,104] Furthermore, antagonists of A_{2B} receptor preferentially inhibits the invasive capacity of breast cancer cells expressing Fos-related antigen-1 (Fra-1), a transcription factor overexpressed in human metastatic breast cancers.^[116] Therefore, the authors suggest that Fra-1 activity is a prognostic indicator of both breast cancer metastasis and responsiveness to pharmacological inhibitors, such as A_{2B} receptor antagonists.^[116]

In a recent paper it has been demonstrated that high expression of A_{2B} receptor is associated with poor survival in triple negative breast cancer (TNBC) patients.^[57] As mentioned above, these authors demonstrate that A_{2B} receptor antagonist prevents metastasis of A_{2B} receptor-expressing tumor cells and improves survival when administered in combination with chemotherapeutic agents and immune checkpoints inhibitors monoclonal antibodies in both experimental and spontaneous murine models of metastasis.^[57] The anti-metastatic effects of A_{2B} receptor antagonists is independent on lymphocytes and myeloid cells, whilst tumor A_{2B} receptor is critical.^[57] These evidence highlight that A_{2B} receptor may be an attractive target for treatment of breast metastasis.

A_{2B} adenosine receptor can also contribute to the pro-angiogenic effects of adenosine in the tumor milieu. Vascular endothelial growth factor (VEGF) is a well-known mediator critically involved in tumor progression and angiogenesis.^[117] A number of studies linked VEGF production to adenosine A_{2B} receptor in human endothelial cells,^[118,119] in some tumor cell lines^[120,121] and in host immune cells, including dendritic cells and myeloid-derived suppressor cells.^[72,80,122]

A_{2B} receptor is expressed on human endothelial cells and its stimulation promotes the expression of several

pro-angiogenic factors, including VEGF, IL-8 and basic fibroblast growth factor (bFGF).^[118] Importantly, under hypoxic conditions the expression of A_{2B} receptor in endothelial and smooth muscle cells increased and the stimulation of these receptors further enhance VEGF release.^[119] Hypoxia is a common feature of tumor and can induce angiogenesis. At the same time, adenosine, whose levels became elevated during hypoxia, further enhances angiogenesis by stimulating A_{2B} receptors, creating a positive feedback between hypoxia, adenosine and VEGF.

Other studies also indicate that adenosine promotes the release of angiogenic factors, namely VEGF and IL-8, in some cancer cells lines, via A_{2B} receptor including human melanoma cells^[120] and glioblastoma cells, which express high levels of A_{2B} receptor under hypoxic conditions.^[121]

Using A_{2B} receptor deficient mice, Ryzhov *et al.*^[72] firstly demonstrated the critical role of A_{2B} receptor in modulating the VEGF levels in tumor tissues. Importantly, vascularization and tumor tissue VEGF levels were significantly reduced in A_{2B} receptor deficient mice compared with WT mice.^[72] This effect was associated with reduced tumor infiltration of VEGF-producing myeloid cells, suggesting that A_{2B} receptor can modulate the release of VEGF either from tumor cells and from host tumor-infiltrating immune cells,^[72] that can contribute to promote tumor angiogenesis.^[123] As mentioned above, adenosine promotes the differentiation of dendritic cells precursors into a subset of DC that produce angiogenic factors, including VEGF, and other immunosuppressive factors via A_{2B} adenosine receptor.^[80] Notably, A_{2B}-stimulated dendritic cells are able to promote tumor growth when injected into mice.^[80] These observations strongly suggest that adenosine sustains tumor angiogenesis during tumor growth by stimulating the release of VEGF from endothelial cells, tumor cells and immune cells. Accordingly, targeting CD73 in mice impairs tumor angiogenesis and decreases VEGF levels, at least in part by lowering adenosine generation in tumor environment that activates A_{2B} receptors.^[124] Therefore, targeting CD73 and/or A_{2B} receptor may represent a potential therapeutic strategy to block angiogenesis. In support of this, the pharmacological blockade of A_{2B} receptor with a selective antagonist in mice significantly reduces the tumor levels of VEGF and CD31 positive cells within tumor lesions.^[122] Moreover, the anti-angiogenic effect of A_{2B} receptor antagonists is, at least in part, dependent on the lower frequency of tumor-infiltrating suppressive myeloid cells (MDSCs),^[72,122] breaking the positive feedback loop that promotes angiogenesis and MDSC-mediated

immune suppression in the tumor environment. Recent evidence indicate that A_{2B} receptor stimulation promotes the release of FGF-2 and C-X-C motif chemokine ligand 12 (CXCL12) from tumor-associated fibroblasts,^[125] that contribute to promote tumor growth and angiogenesis.^[126] These effects are associated with reduced expression of fibroblast activation protein (FAP), a common marker of tumor-activated fibroblasts termed cancer-associated fibroblasts (CAF), that promote tumor growth enhancing tumor immune evasion and tumor vascularization.^[127] A_{2B} receptor-induced CXCL12 by tumor-associated fibroblasts contributes to the pro-angiogenic effects of A_{2B} receptor via CXCR4, suggesting a link between tumor fibroblasts and endothelial cells.^[127] Moreover, fibroblasts express CD73, which is up-regulated under hypoxic conditions.^[127] Altogether, these evidence suggest that in the context of tumor A_{2B} receptor contributes to mediate multiple effects of adenosine on different types of cells that populate the tumor niche. Furthermore, blockade of A_{2B} receptor modulates the intra-tumoral levels of paracrine factors, which are critical in regulating intercellular crosstalk in the tumor microenvironment.

Although the predominant role of A_{2A} receptor in

mediating the immunosuppressive effects of adenosine in the tumor tissue and the high therapeutic potential of blocking adenosine generation and the A_{2A}-mediated effects, by using anti-CD73 monoclonal antibodies and A_{2A} selective antagonists, respectively, it is becoming clear that A_{2B} receptor may significantly affect tumor progression and metastasis. Its contribute to tumor development and growth is most likely dependent on its high expression levels on tumor cells, and/or endothelial cells and/or other tumor-infiltrating cells, in a rich adenosine environment.

CONCLUSION

Adenosine plays a critical role in tumor immunity, angiogenesis and metastasis process. Strategies aimed to inhibit tumor adenosine production and functions, by using CD73 inhibitors and selective blockade of A_{2A} adenosine receptor, are effective for cancer treatments, especially in combination with chemotherapeutic agents and immune-checkpoints inhibitors.

Nonetheless, compelling evidence support the role of A_{2B} receptor subtype in contributing to the pro-tumor effects of adenosine within the tumor

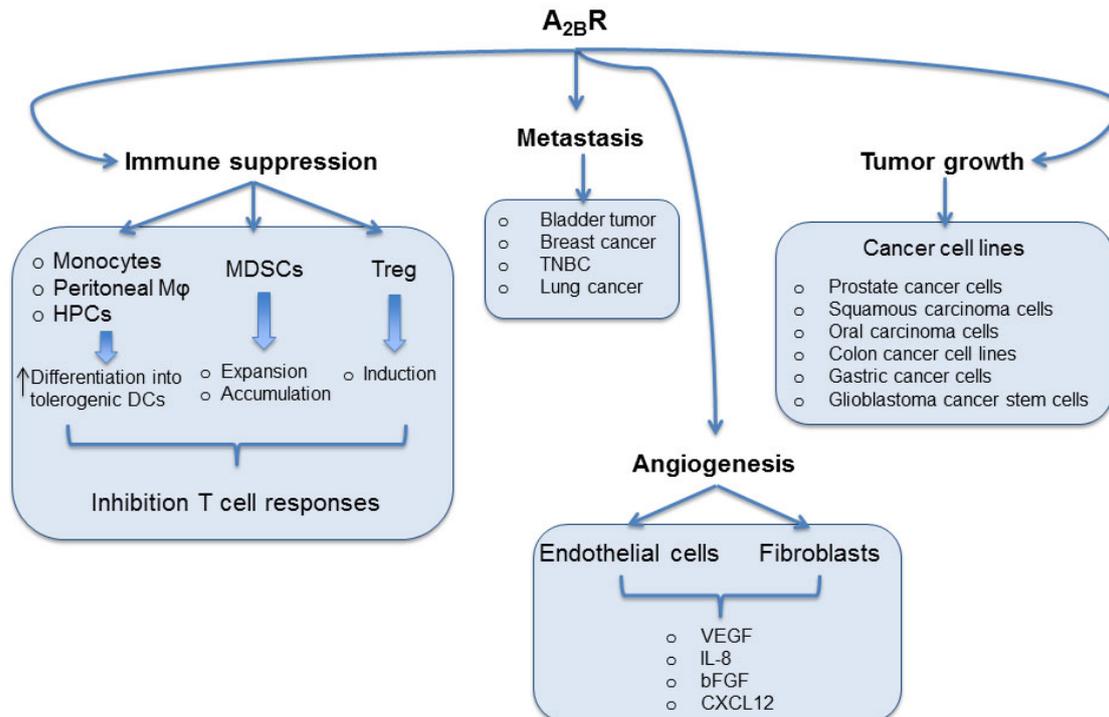


Figure 1: Multiple roles of A_{2B} adenosine receptors in cancer. A_{2B} receptor stimulation induces (1) the differentiation of human monocytes, mouse peritoneal macrophages (φ) and hematopoietic progenitor cells (HPCs) into tolerogenic dendritic cells (DCs); (2) the expansion and accumulation of MDSCs; (3) Treg differentiation, enhancing immune suppression that inhibits T-cell responses. Activation of A_{2B} receptors on stroma cells, including tumor cells, endothelial cells and fibroblasts promotes tumor proliferation or invasion and angiogenesis. TNBC: triple negative breast cancer; VEGF: vascular endothelial growth factor; IL-8: interleukin-8; bFGF: basic fibroblast growth factor; CXCL12: C-X-C motif chemokine ligand 12; MDSCs: myeloid-derived suppressor cells

microenvironment, including immune suppression, angiogenesis and metastasis [Figure 1]. Despite these evidence, further studies are needed to better investigate thoroughly the mechanisms by which blockers of this receptor limit tumor growth. Understanding the relative role of A_{2B} receptor in tumor, depending on the cell types, on its distribution and expression, will help to potentially apply A_{2B} receptor-targeting agents for cancer treatment.

Authors' contributions

Conceived and designed the study: C. Sorrentino, S. Morello

Performed literature search and prepared manuscript: C. Sorrentino, S. Morello

Revised the manuscript: S. Morello

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Conflicts of interest

There are no conflicts of interest.

Patient consent

There is no patient involved.

Ethics approval

This article does not contain any studies with human participants or animals.

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