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Review

Targeting adenosine receptors for the treatment of cancers

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ABSTRACT

Adenosine, produced from the metabolism of adenosine triphosphate (ATP), mediates a number of physiological actions by targeting G protein-coupled receptors, termed adenosine receptors (ARs). Four distinct types of these receptors, namely the A_1, A_{2A}, A_{2B} and A_3AR, have been identified and cloned, each of which shows distinct tissue distribution and function. A number of these receptors are targeted by caffeine and theophylline, ingredients in coffee and tea, which block the effects of endogenous adenosine at these receptor sites and provide the stimulant properties of these beverages. In recent years, a number of laboratories have been studying the role of these receptors in cancers. A number of studies have demonstrated that activation of ARs can either increase or decrease cancer cell proliferation and metastasis. This review will provide evidence implicating ARs in regulating cancer cell growth and proliferation and show how agonists or antagonists for these receptors could mediate anti-cancer responses. The cell signaling pathways mediating the differential responses to activation of AR subtypes will also be discussed.

KEYWORDS: adenosine receptors, angiogenesis, anti-tumor immunity, cancer, metastasis, tumor cell invasion

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INTRODUCTION

Adenosine is a purine nucleoside which signals through a group of widely distributed cell surface guanine nucleotide regulatory (G) protein coupled receptors, adenosine receptors. Four different subtypes of these receptors have been identified and characterized, namely the A_1, A_{2A}, A_{2B} and A_3. The A_{2A} and A_{2B} ARs interact with stimulatory G_s family of G-proteins causing the stimulation of adenylyl cyclase while A_1 and A_3 ARs interact with the inhibitory G_i proteins leading to the inhibition of adenylyl cyclase. The activation or inhibition of adenylyl cyclase respectively results into increase or decrease in the intracellular concentration of cyclic adenosine monophosphate (cAMP). In addition to G_s- and G_i -proteins, ARs are known to signal via other G-proteins to activate other pathways such as phospholipase C (PLC), protein kinase B (PKB), Ca^+ and mitogen-activated protein kinases (MAPKs) like extracellular signal-regulated kinase 1/2 (ERK1/2) [1, 2, 3]. Activation of A_1AR increases PLC activity by inhibiting adenylyl cyclase through pertussis toxin-sensitive G_i-protein. A_1AR is also coupled to the activation of K^+ channels and inactivation of N-, P- and Q-type Ca^{2+} channels [1, 2]. The same appears to be true for A_3AR, which also couples to G_q-proteins to activate PLC. When transiently expressed in COS-7 cells, the A_1AR was shown to activate ERK1/2 via β,γ-subunits released from $G_{i/o}$ G-proteins [4]. Stably expressed A_1AR in Chinese hamster ovary (CHO) cells also activated ERK1/2 [5, 6]. ERK1/2 is a member of MAPK family, which is associated with cell

differentiation, survival, proliferation and cell death [1]. The $A_{2A}AR$ acting through G_{olf} and G_s protein increases cAMP production via activation of adenylyl cyclase which induces inositol 1,4,5-trisphosphate (IP_3) production. The latter promote intracellular Ca^{2+} release and protein kinase C (PKC) activation in COS-7 cells via pertussis toxin-insensitive $G_{\alpha 15}$ and $G_{\alpha 16}$ proteins [1]. $A_{2B}AR$ has lower affinity for adenosine as compared to other ARs and thus require higher concentration of adenosine for its activation [7]. $A_{2B}AR$ is also positively coupled with G_s and G_q proteins. Stimulation of $A_{2B}AR$ activates protein kinase A (PKA) via G_s-protein, whereas it activates PKC via G_q-protein. The $A_{2B}AR$ also activates the arachidonic acid pathway [8]. Thus, the signaling pathway used by the ARs is dictated by the cell type and cell signaling machinery that the cell possesses.

Comparisons of AR expression in tumor versus normal cells have revealed that ARs are differentially expressed in tumor cells and tumor samples. Depending upon the AR expression profile in different tumors, AR subtype specific agonist or antagonist has been used to inhibit tumor growth. The role of A_1AR in tumor growth is highly debatable. A_1AR is highly expressed in MDA-MB-468 breast cancer cells [9] and estrogen receptor α (ERα) positive MCF-7 breast cancer cells [10]. A_1ARs are also highly expressed in colon adenocarcinomatous tissues [11]. $A_{2A}AR$ are over-expressed in A375 melanoma cells [12], MCF-7 breast cancer cells [13], Jurkat T-cell leukemia [14], lung cancer cells [15] and colon cancer cells [16], where it affects cancer cell proliferation, cell death, angiogenesis and anti-tumor immunity. The $A_{2B}AR$ induces angiogenesis in human retinal endothelial cells [17]. These investigators showed that knock down of $A_{2B}AR$ reduced tumor growth and survival time [18]. Inhibition of the $A_{2B}AR$ blocked the release of pro-angiogenic factor interleukin (IL)-8 in A375 human melanoma cells treated with chemotherapeutic agents like etoposide and doxorubicin [19]. Studies have shown that A_3AR is highly expressed in tumor cells such as colon and breast carcinoma [20], A375 melanoma [21], HL-60 leukemia [22], U87MG human glioblastoma [23] and PC-3MM human prostate cancer cells [24] as compared to their normal counterparts. Since induction of A_3AR occurs in several types of cancers [20], this receptor could also serve as a molecular marker for these cancers. This review will describe the expression and functional role of different ARs in the regulation of the tumor growth, angiogenesis, metastasis and anti-tumor immunity.

Adenosine

Cancer cells are metabolically hyperactive with excessive energy and oxygen demands as compared to normal cells. Thus, cancer cells are expected to consume more than the normal amount of ATP and produce higher levels of adenosine. Adenosine exerts its physiological and metabolic effects by acting as a signaling molecule. Adenosine has been designated a 'retaliatory metabolite' [25] because of its acts to protect tissues from different kinds of injury such as ischemia, hypoxia and inflammation [26].

Adenosine is generated and metabolized intracellularly and extracellularly through a stepwise de-phosphorylation of ATP (Figure 1). There are several enzymes that regulate the levels of adenosine production and metabolism. Intracellularly, ATPase along with adenylate kinase converts ATP into adenosine monophosphate (AMP) which is then metabolized by cytosolic-5'-AMP nucleotidase to form adenosine. Adenosine is also produced intracellularly, albeit at lower levels, from S-adenosylhomocysteine (SAH) by SAH hydrolase. CD39 (ecto-ATP apyrase) and CD73 (ecto-5'-AMP nucleotidase) are the extracellular enzymes that convert ATP into AMP and then AMP into adenosine respectively. Adenosine deaminase, which is present intracellularly as well as extracellularly, deaminates adenosine to inosine. In contrast, adenosine kinase, a cytosolic enzyme, catalyses the formation of AMP from adenosine [27]. These two enzymes thus utilize adenosine and lower its concentration.

Adenosine is a relatively hydrophilic molecule, which requires specialized transporters to cross the cell membrane. Two different nucleoside transporters have been identified that maintains adenosine homeostasis across the cell membrane. Concentrative nucleoside transporter (SLC28) is found only in specialized epithelial cells and

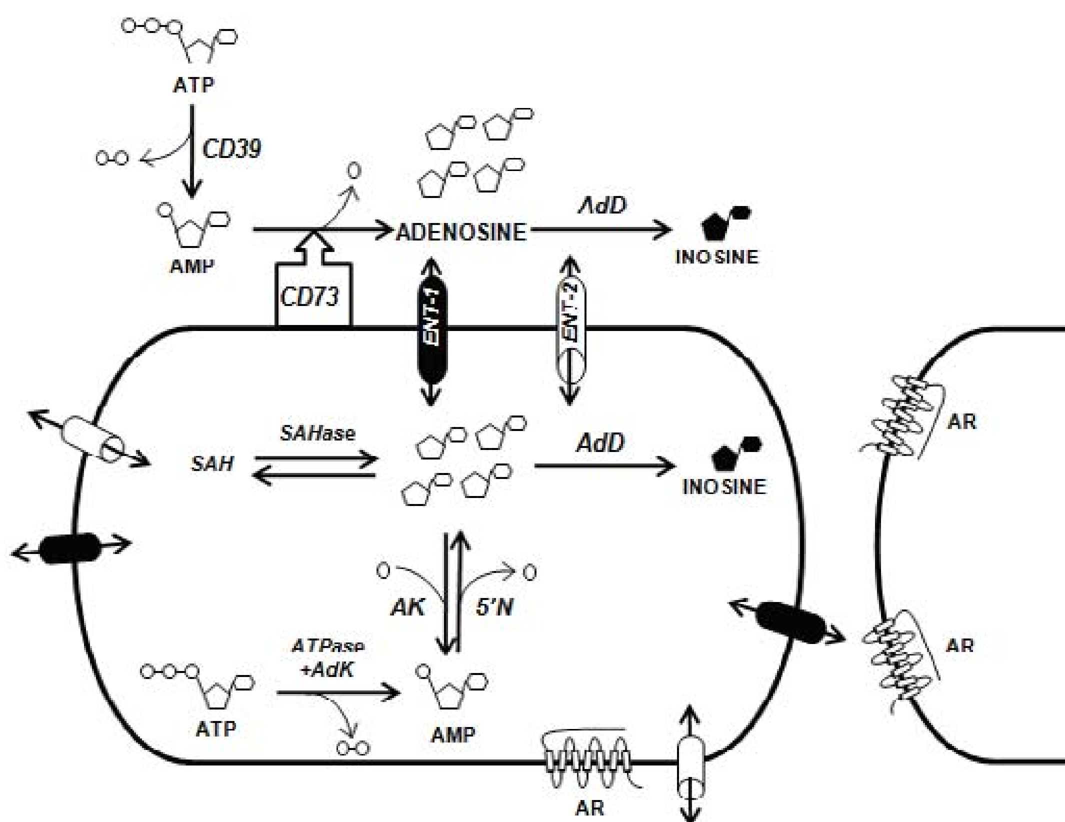

Figure 1. Adenosine metabolism. Extracellularly, adenosine is formed from adenosine triphosphate (ATP) by ecto-ATP apyrase (CD39) and ecto-5'-AMP nucleotidase (CD73). Intracellularly, adenosine is produced by ATP hydrolyzing enzymes (ATPase), adenylyl kinase (AdK) and cytosolic-5'-AMP nucleotidase (5'N) and from S-adenoslyhomocysteine (SAH) by SAH hydorlase (SAHase). Once produced, adenosine is transported in or out of the cells by equilibrative nucleoside transporters (ENT-1 and ENT-2). Adenosine deaminase (AdD) and adenosine kinase (AK) are the two enzymes that convert adenosine into inosine and adenosine monophosphate (AMP) respectively. Extracellular adenosine acts on adenosine receptors (AR) and initiates a cascade of intracellular signaling. Symbols: ○, one phosphate molecule; ⬠, ribose molecule; ⬭, adenine nucleotide; ⬟, inosine; ⬬, ENT-1; ⊂⊃, ENT-2; , adenosine receptors.

involves active Na^+-dependent nucleoside transport. Equilibrative nucleoside transporter (ENT) family (SLC29) is a bidirectional, ubiquitous passive nucleoside transporter which maintains the concentration gradient of adenosine across the cell membrane [28, 29]. Four ENT isoforms are found in human and rodent genome, of which ENT-1 and ENT-2 are the best studied. Dipyridamole, a coronary vasodilator and cardio-protectant, inhibits ENTs to prevent the re-uptake of adenosine into the cells, thereby increasing extracellular adenosine concentrations and AR activation [28].

Evidence suggests that adenosine release during hypoxia is inversely proportional to O_2 levels [30, 31]. This is because the activities of most of the enzymes and transporters that are involved in adenosine production, metabolism and release are differentially regulated under hypoxic environment. Studies in rat pheochromocytoma (PC12) cells reported that hypoxia decreased the expression and activities of adenosine utilizing enzymes, adenosine kinase and adenosine deaminase, in a time dependent manner, thus increasing the availability of adenosine in the extracellular milieu [31]. This study also indicated that chronic hypoxia up-regulated adenosine producing enzymes such as ecto- and cytosolic-5'-AMP nucleotidase as well as SAH hydrolase. The induction of CD73 in hypoxia has been shown to occur in hypoxia-inducible factor (HIF)-1-dependent manner [32].

Moreover, hypoxia is also known to down-regulate the adenosine transporter, ENT-1 expression in PC12 [31] and endothelial cells [33] via HIF-1-dependent transcriptional repression and in mouse cardiomyocytes via PKC mechanism [34]. Interestingly, ENT-2 was down-regulated in mouse cardiomyocytes, but unlike ENT-1, its expression was not affected by hypoxia in PC12 [31] and endothelial [33] cells. Thus, hypoxia appears to increase generation of adenosine both intra- and extracellularly.

ARs: Effect on cancer cell proliferation and cell death

One of the most important characteristics of the cancer cells is that they show rapid growth in comparison to the normal cells. Hence one of the ways that tumor growth can be inhibited is by suppressing the cancer cell proliferation or enhancing their killing.

A_1AR

Breast cancer tissues show high expression of A_1AR which promote tumor cell growth. Selective knock down of A_1AR by small interfering (si) RNA in MDA-MB-468 breast cancer cells reduced proliferation and increased cell death. These cells were arrested in G2/M phase due to impaired G1 checkpoint and resulted from increased expression of cyclin-dependent kinase (CDK) inhibitor p27 along with decreased CDK4 and cyclin E expression [9]. The expression of A_1AR in estrogen receptor (ER)-α positive breast tumors is dependent on the activation of estradiol-ERα axis [10]. siRNA-mediated reduction of A_1AR in ERα+ cells (MCF-7) suppressed the proliferation of these cells and reduced expression of ERα [35]. These findings support the presence of a positive feedback mechanism in which the expression of A_1AR is increased by estradiol-ERα, while the A_1AR stimulates the ERα expression in breast cancer cells. Thus, it appears that A_1AR is pro-tumorigenic in breast cancer. In contrast, A_1AR activation has anti-tumor effects in colon cancer. Like breast cancer tissues, these receptors are over-expressed in colon adenocarcinomatous tissues as compared to the normal peri-tumoral tissues [11]. However, treatment of HT29 and CW2 colon cancer cells with an A_1AR agonist reduced cell proliferation and increased cell death, respectively [36, 37]. The adenosine-induced cell death involved activation of caspase-3, -8 and -9 and was mimicked by an adenylate cyclase inhibitor, suggesting that increased cAMP levels in these cells is responsible for their tumorigenicity [37]. Similarly, the A_1AR-mediated activation of caspase-3 and -9 was also involved in the death of astrocytoma cells through a cAMP dependent mechanism [38].

A_{2A}AR

Using a high-throughput screening process, it was demonstrated that A_{2A}AR agonist synergizes with phosphodiesterase (PDE) inhibitors to suppress cell proliferation and induce cell death in B-cell lymphomas [39]. This synergistic action was seen in both glucocorticoid -sensitive and –insensitive multiple myeloma cells and involved the production of cAMP. The PDE4B isoform appears to be the most important target as it is up-regulated in presence of high cAMP levels [39]. The synergistic actions of A_{2A}AR agonist and PDE inhibitors in combination with glucocorticoids opens up a new therapeutic option against B-cell lymphomas (multiple myeloma and diffuse large B-cell lymphoma) that can increase the effectiveness of these drugs at lower concentrations, thus reducing the untoward effects seen with the use of high-dose therapies. The anti-proliferative and pro-apoptotic effects of A_{2A}AR activation were also seen in the colon cancer cells [16]. The A_{2A}AR agonist disrupted the mitochondrial membrane potential and induced the activation of the caspase-9 and -3 (but not caspase-8), thus increasing the killing of Caco-2 human colon cancer cells. This A_{2A}AR-mediated action was dose- and time-dependent, and was also associated with activation of adenyl cyclase enzyme. Accordingly, these effect were mimicked by forskolin (activator of adenylyl cyclase) while inhibited by A_{2A}AR antagonist and an adenylate cyclase blocker [16]. In addition to the classical A_{2A}AR signaling pathway, the pro-apoptotic effects of A_{2A}AR agonists also involve the non-adenylate cyclase-cAMP axis. In melanoma cells, for example, activation of A_{2A}AR promoted cancer cell death that was mediated via the PKC-ERK1/2 pathway [12].

Unlike anti-tumor effects described above, $A_{2A}AR$ agonist enhanced the proliferation of hormone-dependent MCF-7 breast cancer cell line by increasing the transcription of the progesterone receptor. Furthermore, $A_{2A}AR$ antagonist reduced the ethanol-mediated activation of ER-α signaling pathway, suggesting a cross-talk between $A_{2A}AR$ and ER-α in mediating proliferative effects of ethanol in MCF-7 cells [13]. Thus, the direct effect of $A_{2A}AR$ on tumor cell growth might depend on the type of cancer.

$A_{2B}AR$

$A_{2B}ARs$ are over-expressed in colorectal carcinoma tissues and colon cancer cell lines as compared to the normal colorectal mucosa. The expression of this receptor was increased by hypoxia. Activation of $A_{2B}AR$ increased proliferation of the colon cancer cells [40]. Apart from colon cancer cells $A_{2B}ARs$ were also detected in ER negative breast cancer cells, MDA-MB-231, where activation of these receptors increased adenylyl cyclase activity and increased intracellular Ca^{2+} release via PLC-mediated signaling pathway [41].

A_3AR

Most studies have documented an anti-cancer role for A_3AR agonists. However, some studies have also shown the usefulness of A_3AR antagonists against cancer cells. One of the earliest studies elucidating the tumor suppressive role of adenosine showed that skeletal muscles are resistant to tumor metastases due to the presence of adenosine present in conditioned medium obtained from cultured cells [42]. Later it was shown that this anti-tumor activity was mediated primarily through the A_3AR [43]. Subsequently, A_3AR has been shown to inhibit the growth of lymphoma [44], melanoma [43], colon cancer [45], prostate cancer [46], hepatoma [47], breast cancer [48], leukemia [49], lung cancer [50], glioblastoma [51] and mesothlioma [52] cells. The A_3AR-mediated tumor-suppressive effects involved inhibition of cancer cell proliferation, cell cycle arrest and increased cell death. The classical pathway demonstrated includes activation of the A_3AR with subsequent inhibition of cAMP production, inhibition of PKA and PKB with resulting inhibition of glycogen synthase kinase (GSK)-3β activity and destabilization of β-catenin. Additionally, the A_3AR reduces nuclear factor (NF)-κB activity in a cAMP-PKA-PKB-dependent manner. Both of these pathways led to suppression of cyclin D1 and *c-myc*, cell cycle arrest and/or apoptosis, and eventually tumor growth inhibition [53].

Although the A_3ARs have primarily been targeted as inhibitors of the tumor growth, some studies have shown that the A_3AR promotes tumor angiogenesis and increase in tumor growth. These studies have opened a new debate on the use of A_3AR antagonists as the anti-tumor agents, and are described in detail in following section.

ARs: effect on tumor angiogenesis

Tumor growth *in vivo* ceases once it reaches the size of ~1 – 2 mm in diameter. To grow further the tumor requires its own blood supply. The recruitment of the blood vessels by the tumor cells is referred to as tumor angiogenesis. Endothelial cells form the inner lining of the blood vessels and its proliferation, migration and tube formation are the key events in angiogenesis [27]. Angiogenesis is a result of the tumor-vascular cell interaction. Under favorable conditions, pro-angiogenic growth factors are secreted by the tumor cells which bind to its receptors on endothelial cells. The endothelial cells then proliferate and migrate through the extracellular matrix towards the tumor in response to different pro-angiogenic growth factors and cytokines.

The angiogenic response of the endothelial cells is important for the tumor cells to survive under conditions of oxygen deprivation (hypoxia). Angiogenesis is also important for the tumor cells to invade the surrounding tissue and metastasize to a distant organ to form a secondary tumor.

It has been long known that adenosine plays an important part in stimulating angiogenesis in physiological or pathological conditions [27, 54]. Activation of specific adenosine receptors have been shown to induce wound healing and angiogenesis. The exact mechanism by which adenosine stimulates angiogenesis is poorly understood. However, many studies have shown that all four adenosine receptor subtypes coordinate and participate in mediating the angiogenic actions of adenosine [27, 55]. The presumed

mechanism of adenosine-induced angiogenesis under hypoxic conditions involves the release of vascular endothelial growth factor (VEGF) following the activation of ARs present on parenchymal cells by extracellular adenosine [17, 27]. The parenchymal cells could be any cell or tissue which is subjected to hypoxia. VEGF, released from the parenchymal cells, stimulates proliferation and migration of endothelial cells inducing angiogenesis [56] which could result in tumor growth. The AR subtype which induces VEGF production depends on species and cell type [27].

A_{2A}AR and A_{2B}AR

In retinal endothelial cells, A_{2A}AR and A_{2B}AR stimulation induced cell proliferation and VEGF gene expression in PKA dependent manner [17, 57]. Activation of A_{2B}AR expressed in human microvascular endothelial cells (HMEC-1) increased the expression of VEGF, in addition to other pro-angiogenic factors, such as IL-8 and basic fibroblast growth factor (bFGF) [58]. Desai *et al.* [59] demonstrated that stimulation of A_{2A}AR in human microvascular endothelial cells (HMVEC) led to increased vascular tube formation by inhibiting the release of anti-angiogenic factor, thrombospondin-1. Activation of the A_{2B}AR also promoted the proliferation of human umbilical vein endothelial cells by activating a cAMP-dependent, but PKA-independent pathway involving Epac1 (exchange protein activated by cAMP-1) [60]. Thus, these AR subtypes appear to participate in the release of VEGF in a direct or indirect manner in non-cancerous cells.

Reports of AR-stimulated angiogenesis in cancer models have been limited. Working on the previous findings that tumor-associated immune cells promote the progression of tumors [61, 62], Ryzhov *et al.* [18] hypothesized that activation of A_{2B}AR located on tumor-infiltrating host immune cells could produce an angiogenic response. Using an A_{2B}AR knock-out mouse model, this study demonstrated that adenosine activation of A_{2B}AR located on tumor-infiltrating host immune cells increased the release of VEGF from these cells, increased intratumoral vessel density and promoted Lewis lung carcinoma growth. Hypoxia is the hallmark of cancer and a key regulator of angiogenesis. For example, in the core of human glioblastoma, the oxygen concentration can be as low as 5 mm Hg (~ 0.7 % O_2) [63]. In hypoxic human melanoma cells the A_{2B}AR antagonists reduced IL-8 production in presence of etoposide and doxorubicin [19]. Adenosine also increased the release of IL-8 from hypoxic U87MG cells via the A_{2B} receptors [64]. Thus, blocking A_{2A}- and A_{2B}-AR subtypes by specific antagonists alone or in combination with chemotherapeutic agents may inhibit VEGF production and ultimately reduce angiogenesis. Some studies have also reported HIF-dependent induction of AR subtypes in hypoxia. Human A_{2A}AR and A_{2B}AR promoters possess functional binding site for HIF-2α and HIF-1α, respectively [15, 65]. The transcriptional activation of both of these receptors promoted hypoxia-induced angiogenesis in endothelial cells [65] including human lung microvascular endothelial cells [15]. This could account for enhanced growth of lung cancer cells where A_{2A}AR are highly expressed compared to adjacent normal lung tissue [15].

A_3AR

A_3AR can modulate HIF-1α-dependent VEGF expression by up-regulating HIF-1α. HIF-1α is one of the major transcription factors regulated under hypoxic conditions. Activation of HIF-1α under hypoxic conditions induces the transcription of hypoxia-responsive genes which mediate tumor angiogenesis (such as VEGF), tumor invasion, survival and metabolic activity [66]. In hypoxia, A_3AR activation led to the accumulation of HIF-1α in melanoma, glioblastoma, osteosarcoma, colon carcinoma, ovarian carcinoma and breast carcinoma cells [67]. It was further established that A_3AR-induced HIF-1α and VEGF required activation of ERK1/2 and p38 MAPK in hypoxic glioblastoma cells [23], while blockade of A_3AR reduced VEGF production in A375 human melanoma cells treated with etoposide and doxorubicin [19]. Adenosine can also stimulate endothelial cell proliferation independent of VEGF. This mechanism may involve changes in other pro- or anti-angiogenic growth factors. Studies on A375 melanoma cells show that the selective A_3AR agonist induced accumulation of HIF-1α in hypoxia which significantly increased the expression of pro-angiogenic factor *Ang-2*

(but not *VEGF*). This response was regulated via the ERK1/2 and p38 MAPK pathways [67].

ARs: effect on tumor cell migration and invasion

Tumor metstasis is an organized event which involves migration of tumor cells from their primary harboring sites to distant organs. The process of cell motility is influenced by autocrine and paracrine factors. One such factor is adenosine. Adenosine promotes chemotaxis in neutrophils [68] and endothelial cells [69] and can increase or suppress the invasiveness of the cancer cell (as described below).

A_1AR

Adenosine and AMP increased the migration of melanoma cells in the absence of any exogenous chemotactic agents. This effect was mimicked by agonists and inhibited by antagonists specific for the A_1AR [70] which suggest that this receptor is a viable target for developing anti-metastatic therapies. However, unlike melanoma, activation of A_1AR suppressed the glioma-stimulated release of matrix metalloproteinase (MMP)-2 by microglial cells [71]. Gliomas are one of the most invasive tumors of CNS, and microglia play an important role in the glial cell invasion [72]. Microglial cells are important source of MMP-2, which increase the infiltrative behavior of glioma cells [73]. Thus, inhibition of MMP-2 activity by an A_1AR agonist suggests the anti-invasive effect of these receptors in gliomas. Thus, the A_1AR effects on tumor cell invasion might be dependent on the type of cancer.

A_{2B}AR

Activation of the A_{2B}AR promotes tumor cell migration and invasion of breast cancer and colon cancer cells. Breast cancer cells show increased expression of *CD73* (an ecto-5'-nucleotidase) [74]. CD73 is primarily responsible for catalyzing dephosphorylation of extracellular AMP to adenosine [75]. The increase in adenosine promotes cancer cell migration and invasion. This pro-invasive action of adenosine was mimicked by A_{2B}AR-specific agonist and blocked by its antagonist. Although, there was involvement of A_{2B}AR in the metastasis of breast cancer cells *in vivo*, treatment of mice with A_{2B}AR agonist did not affect the growth of the tumor [76]. Activation of A_{2B}AR increased the invasiveness of the deleted in colon cancer (DCC)-deficient human colon cancer cells [77]. This action of A_{2B}AR was dependent on the PKA which regulated the activity of Rho GTPase and Rho-kinase [77]. The Rho-Rho kinase axis is important for the activation of cytoskeleton proteins of the cells, responsible for movement and migration of the cancer cells [78].

A_3AR

The direct actions of A_3AR agonists on the cancer cells suppress their migration and invasion, thereby reducing metastasis. Our laboratory previously demonstrated that the A_3AR reduced lung metastases of the prostate cancer cells in a xenograft mouse model [24]. This anti-metastatic action involved A_3AR-mediated reduction in reactive oxygen species (ROS) via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. We showed that prostate cancer cells demonstrate high NADPH oxidase activity. Knockdown of $p47^{phox}$ and Rac1, subunits essential for the activity of NADPH oxidase, reduced invasiveness of prostate cancer cells [24]. The molecular mechanism underlying A_3AR agonist-mediated inhibition of NADPH oxidase involved inhibition of adenylyl cyclase with subsequent reductions in cAMP/PKA activity. The suppression of cAMP/PKA axis led to reduced activation of ERK1/2, reduced phosphorylation of $p47^{phox}$ and ultimately to inhibition of NADPH oxidase activity [24]. In glioblastoma cells, however, A_3AR activation led to increased ERK1/2, c-Jun N-terminal kinase/stress-activated protein kinase (pJNK/SAPK) and PKB activities. This increased MMP-9 and invasiveness of these cells [51].

ARs: effect on anti-tumor immunity

Tumor microenvironment demonstrates the infiltration of the immune cells, which have come into focus as the promoters of the tumor growth by helping cancer cells evade anti-tumor immunity [79]. In addition to the tumor-associated immune cells, cancer cells themselves can suppress anti-tumor immune response by elaboration of immunosuppressive small molecules, such as

transforming growth factor-β [80], IL-10 [81], prostaglandin E2 (PGE2) [82] and adenosine [83].

A_{2A}AR

The A_{2A}AR is the main focus of research studies focusing on the immunosuppressive role of adenosine in cancer. Activation of the A_{2A}AR enhances tumor progression by inhibiting the function of anti-tumor immune cells, such as T-lymphocytes and lymphokine-activated killer (LAK) cells [84]. A_{2A}ARs are present on CD4+ and CD8+ T lymphocytes, and LAK cells which infiltrate the tumor microenvironment [85, 86, 87]. Activation of the A_{2A}AR inhibited anti-tumor T cell activity thereby promoting tumor growth [88, 89]. In A_{2A}AR-deficient mice, immunogenic melanoma cells elicited an autoimmune response with complete rejection of the cancer cells in ~60% of the mice [90]. The A_{2A}AR-mediated inhibition of T-lymphocytes and LAK cells involved increased generation of cAMP and activation of PKA [91, 92]. Anti-melanoma specific CD8+ and CD4+ T-cells activity was suppressed by A_{2A}AR-specific agonist, and this involved activation of the PKA-I regulatory subunit as blockade of catalytic subunits of PKA did not reduce the adenosine effects while the PKA-I blocker suppressed the adenosine response. Adenosine also reduced the production of cytokines, such as interferon (IFN)-γ, IL-2 and tumor necrosis factor (TNF)-α [91]. In addition to the cAMP/PKA pathway, the A_{2A}AR-dependent response was also complimentary to the PGE2 inhibitory response in LAK cells [92, 93]. This co-operative response of adenosine and PGE2 involved increased CREB phosphorylation and inhibition of Akt, along with amplification of the cAMP-PKA signaling [93]. The adenosine and PGE2-dependent action also led to the activation of Csk, an inhibitor of the T-cell receptor (TCR) signaling [94]. Upon activation, Csk inhibited Lck, ZAP-70 and phosphorylated Akt. The co-operative action of adenosine and PGE2 also resulted in lack of inhibition of the tumor infiltrating lymphocytes following pre-treatment with sub-suppressive doses of adenosine or PGE2, which could be due to cross desensitization of the PGE2 and ARs. This process might be useful in adoptive immunotherapy as it can improve the effectiveness of the anti-tumor immune cells by pre-treating them with low doses of PGE2 or A_{2A}AR agonist before their adoptive transfer [94]. However, in contrast to the above mentioned mechanisms, A_{2A}ARs can also enhance the anti-tumor immunity by suppressing the activation-induced cell death (AICD) of the CD4+ T cells [95]. AICD results from TCR-mediated stimulation of the already activated T-cells that involves interaction of Fas and Fas ligand [96]. A_{2A}AR-specific agonist reduced the expression of Fas and Fas ligand and inhibited the apoptosis of CD4+ cells by AICD. This PKA-dependent process also involved the suppression of transcription factors, such as NF-κB, nuclear factor of activated T cells (NF-AT) and early growth response (Egr)-1 and -3 proteins [95]. Thus, A_{2A}AR-mediated reduced killing of CD4+ T cells could indirectly promote their immune response. This action of A_{2A}AR is just opposite of the inhibitory actions of A_{2A}AR on T-lymphocytes and LAK cells that promote tumor growth. So far, anti-AICD functions of A_{2A}ARs have not been described in tumor cells, but future studies should address this and determine whether this can influence the pro-tumor action of A_{2A}ARs.

A_{2B}AR

A_{2B}ARs have also been implicated in the adenosine-mediated inhibition of anti-tumor immunity [90]. Mice lacking A_{2B}ARs (A_{2B}AR-KO) showed reduced tumor growth of the Lewis lung cancer cells along with prolonged survival time of these mice [18]. Although the number of CD45+ immune cells infiltrating the tumor site was not significantly different in A_{2B}AR-KO mice as compared to the wild-type mice, the VEGF secretion by these cells was low in A_{2B}AR-KO mice [18]. This suggested that the A_{2B}AR promotes cancer cell growth by increasing VEGF production by tumor-associated immune cells.

A_1AR

A_1ARs are widely expressed in brain with a potential impact on various pathological conditions of brain and central nervous system. These pathological conditions also include different types of gliomas, like globalstoma and astrocytoma [97]. In fact, the expression of A_1ARs is increased in the immediate vicinity of the invasive gliomas [98]. In addition to the neurons, A_1ARs are also highly expressed in

microglia, native immune cells of the brain [99]. In an elegantly done study, Synowitz *et al*. [71] showed the inhibitory role of A_1AR in the development of glioma was dependent on the presence of microglial cells. A_1AR-deficient mice had larger Gl268 glial cell tumor burden as compared to their wild-type counterparts. Additionally, these mice also demonstrated high infiltration of microglia around the tumor. The microglia-dependent suppression of glioma tumor growth by A_1AR was confirmed in organotypical brain slice cultures where glioma cells were grown in presence or absence of microglial cells. The microglia-devoid cultures showed enhanced tumor growth even in presence of A_1AR agonist while cultures with intact microglial cells were less proliferative under similar treatment conditions [71]. Thus, by modulating the activity of immune cells in the tumor microenvironment, A_1AR activation can regulate the aggressive growth of glioma cells. The development of A_1AR agonists as immunomodulator anti-glioma drugs will require future studies detailing the molecular mechanisms involved not only in microglial inhibition but also in the development of gliomas.

A_3AR

Immune cells, including tumor-infiltrating anti-tumor cells, also express A_3ARs [84]. Similar to the A_1AR, A_3AR activation increases the anti-tumor immune response. In a murine melanoma model, A_3AR agonist increased the infiltration and activation of the natural killer (NK) cells in the melanoma tissues with increased IL-12 production. IL-12 is a known activator of NK cells and is also involved in the anti-tumor activity as it exerts cytotoxic effects against the tumor cells [100]. Furthermore, adoptive transfer of immunity to recipient mice inoculated with splenocytes from the A_3AR agonist-treated mice, led to reduced number of metastatic foci in the lungs [101]. In a similar fashion, A_3AR agonist suppressed colon carcinoma growth and liver metastases by enhancing the NK cell activity and IL-12 secretion [45]. In a recent study, Morello *et al*. [102] showed that A_3AR agonist also increase the recruitment of the CD8+ T cells in the melanoma tumor microenvironment. This was associated with increased production of TNF-α and IFN- γ. The killing of melanoma cells was dependent on these anti-tumor immune cells, as the mice in which NK and CD8+ T cells were depleted failed to show tumor suppressive effects of A_3AR agonist [102]. Thus, in addition to the widely studied direct effects of A_3AR activation on tumor cells, these receptors can also influence the tumor growth via an indirect mechanism involving the immune system.

CONCLUSION

ARs appear to be viable targets for the development of anticancer drugs. Activation of these receptors has been shown to possess either anti-cancer or oncogenic actions. Thus, selective agonists or antagonists of these receptors could serve as effective anticancer agents. Future studies are expected to clearly further delineate a direct versus an indirect role (immune cell activation) of ARs in the regulation of tumor growth and metastasis.

ABBREVIATIONS

$A_{2B}AR$-KO	:	$A_{2B}AR$-knock out
AdD	:	adenosine deaminase
AdK	:	adenylate kinase
AICD	:	activation-induced cell death
AK	:	adenosine kinase
AMP	:	adenosine monophosphate
AR	:	adenosine receptor
ATP	:	adenosine triphosphate
bFGF	:	basic fibroblast growth factor
cAMP	:	cyclic adenosine monophosphate
CDK	:	cyclin-dependent kinase
Egr	:	early growth response
ENT	:	Equilibrative nucleoside transporter
ERK1/2	:	extracellular signal-regulated kinase 1/2
ERα	:	estrogen receptor α
GSK-3β	:	glycogen synthase kinase-3β
HIF	:	hypoxia-inducible factor
IFN-γ	:	interferon-γ
IL	:	interleukin
LAK	:	lymphokine-activated killer
MAPK	:	mitogen activated protein kinase
MMP	:	matrix metalloproteinase
NADPH	:	nicotinamide adenine dinucleotide phosphate
NF-κB	:	nuclear factor-κB

NFAT	:	nuclear factor of activated T cells
NK	:	natural killer
IP3	:	inositol 1,4,5-trisphosphate
PDE	:	phosphodiesterase
PGE2	:	prostaglandin E2
pJNK/SAPK	:	c-Jun N-terminal kinase/stress-activated protein kinase
PLC	:	phospholipase C
PKA	:	protein kinase A
PKB	:	protein kinase B
PKC	:	protein kinase C
ROS	:	reactive oxygen species
SAH	:	S-adenosylhomocysteine
siRNA	:	small interfering RNA
TCR	:	T-cell receptor
TNF-α	:	tumor necrosis factor-α
VEGF	:	vascular endothelial growth factor

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