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Targeting the hypoxia-adenosinergic signaling pathway to improve the adoptive immunotherapy of cancer

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Abstract

The recent approval by the FDA of cancer vaccines and drugs that blockade immunological negative regulators has further enhanced interest in promising approaches of the immunotherapy of cancer. However, the disappointingly short life extension has also underscored the need to better understand the mechanisms that prevent tumor rejection and survival even after the blockade of immunological negative regulators. Here, we describe the implications of the “metabolism-based” immunosuppressive mechanism, where the local tissue hypoxia-driven accumulation of extracellular adenosine triggers suppression via A2 adenosine receptors on the surface of activated immune cells. This molecular pathway is of critical importance in mechanisms of immunosuppression in inflamed and cancerous tissue microenvironments. The protection of tumors by tumor-generated extracellular adenosine and A2 adenosine receptors could be the misguided application of the normal tissue-protecting mechanism that limits excessive collateral damage to vital organs during the anti-pathogen immune response. The overview of the current state of the art regarding the immunosuppressive effects of extracellular adenosine is followed by an historical perspective of studies focused on the elucidation of the physiological negative regulators that protect tissues of vital organs from excessive collateral damage, but, as a trade-off, may also weaken the anti-pathogen effector functions and negate the attempts of anti-tumor immune cells to destroy cancerous cells.

Keywords

adenosine; A2A adenosine receptor; cyclic AMP; hypoxia; inflammation; tumor; cancer immunotherapy; adoptive immunotherapy; tumor microenvironment; immunosuppression; T lymphocytes; regulatory T cells; cytokines; cytotoxicity

Neutralization of immunosuppressive mechanisms is necessary for successful cancer immunotherapy

Among current approaches to the immunotherapy of cancer are i) the use of cancer vaccines to stimulate *in vivo* development and expansion of anti-tumor T cells that are capable of recognizing and destroying tumor cells [1, 2], ii) blockade of immunological negative regulators [3, 4], and iii) adoptive immunotherapy of cancer, which involves preparation and expansion of patients' own [5, 6] or modified [7] tumor reactive T cells *in vitro* and their subsequent infusion into the same patient. The recent approval by the FDA of cancer vaccines and drugs that blockade immunological negative regulators has further enhanced

interest in these promising approaches [4, 8, 9]. However, the disappointingly short life extension has also underscored the need to better understand mechanisms that lead to tumor rejection as well as mechanisms that prevent better survival even after the blockade of immunological negative regulators.

Here we will review the current state of the art regarding the immunosuppressive effects of extracellular adenosine and a historical perspective of studies focused on elucidation of the physiological negative regulators that may negate the attempts of anti-tumor immune cells to eliminate cancerous cells. These studies have been motivated by the old-standing paradoxical observations that cancer patients do have tumor-reactive blood cells that were capable of destroying tumor cells *in vitro*, but were not capable of destroying cancer cells *in vivo* [10]. This was explained by multiple mechanisms of immunosuppression in the tumor microenvironment (TME) since tumor cells are surrounded *in vivo* by regulatory T cells, myeloid-derived suppressor cells, tolerogenic cytokines and inhibitory cell surface molecules [11–13].

There are also implications of the “metabolism-based” immunosuppressive mechanisms. These mechanisms inhibit anti-tumor immune cells and thereby protect cancerous tissues by inhibiting the proliferating immune cells by limiting their access to nutrients due to e.g. indoleamine 2,3-dioxygenase-mediated depletion of the essential amino acid [14] or by directly inhibiting the immune cell-activating signal transduction by accumulated ligands of immunosuppressive receptors in the TME [15, 16]. The identification of tumor protecting immunosuppressive ligands and receptors was guided by an assumption that the immunosuppressive mechanism in the TME is the misguided application of the same mechanism that protects normal tissues from excessive collateral damage during the anti-pathogen immune response. These assumptions led to the identification of the critical importance of the local tissue hypoxia-driven accumulation of extracellular adenosine and of A2A adenosine receptor (A2AR) on the surface of immune cells in mechanisms of immunosuppression [15–17]. The adenosine-A2AR pathway represents a part of the physiological mechanism of immunosuppression that is complementary to other immunological negative regulators.

Endogenous immunoregulator prevents collateral damage to normal tissues during inflammation

It was a long-standing belief that cytotoxic T cells, which form conjugates with the antigen bearing target cells in response to the recognition of the antigen, destroy only the antigen-bearing target cell without attacking the normal cells located in the vicinity of target cells, or “innocent bystanders”. However, while the cytotoxic granule exocytosis by effector cytotoxic T cells in a conjugate is not likely to cause collateral damage [18], other effector functions of immune cells such as secretion of pro-inflammatory cytokines could be damaging to innocent bystander cells [19]. Therefore, it was important to understand why the vast majority of infected patients do recover with no serious complications after anti-pathogen immune cells destroyed the pathogen, even when there is a possibility of excessive collateral damage to normal tissues [20]. The likely explanation was the existence of an immunosuppressive mechanism that evolved to protect normal tissues from collateral damage during anti-pathogen immune response [20].

Intracellular cAMP was known to be immunosuppressive for a long time and it was also established that cAMP is a high fidelity immunosuppressor. Studies of the inhibitory action of intracellular cAMP in T cell receptor-triggered signaling established that cAMP inhibits both early and late stages of trans-membrane signaling pathway [21]. Thus, the intracellular cAMP was a good candidate to function in lymphocytes as the intracellular

immunosuppressor and protector of normal tissues from excessive collateral damage. It was suggested to use inhibitors of cAMP-dependent protein kinase to block inhibition and modulate T cell receptor-triggered activation of cytotoxic T-lymphocytes [22]. However, this was not feasible due to the redundancy of expression of different catalytic and regulatory subunits of cAMP-dependent protein kinase and the complexity of the drug design [23].

A much more attractive strategy to prevent the intracellular cAMP-mediated immunosuppression was to antagonize those Gs protein-coupled receptors that trigger the accumulation of intracellular cAMP in inflamed and cancerous tissues. However, there are many Gs protein coupled receptors that trigger the accumulation of intracellular cAMP [24] and it was critical to establish conclusively which of many Gs protein coupled receptors –if any—are likely to be inhibiting immune cells *in vivo*. These ligands include adenosine, adrenaline (β -adrenergic receptors), dopamine (D1 and D5 receptors), histamine (H2 receptor), prostanoids (DP, IP, EP2 and EP4 receptors), serotonin (5-HT₄, 5-HT₆, 5-HT₇ receptors) and some small peptides (PAC1, VPAC1, VPAC2 and glucagon receptors).

A local modulator of immune responses would likely increase in concentration in response to metabolic changes in inflamed and cancerous tissues. We focused on considerations of the cyclic AMP-elevating A_{2A} and A_{2B} adenosine receptors [25] with a special focus on the high affinity A_{2A} [26–30]. Subsequent studies demonstrated that T cells do express A_{2A} [26–28] in a gene dose-dependent manner [29] and, importantly, there is no A_{2A} reserve, i.e. there are no spare A_{2A}.

Pharmacological studies to support the immunosuppressive role of the extracellular adenosine-A_{2A} pathway

There is a long history of studies of A_{2A} (reviewed by [31]) by neurobiologists, cardiovascular biologists and pharmacologists [32, 33], which was fueled in part by an interest in the mechanisms of action of caffeine [34, 35] and the mechanisms of Parkinson's disease [36]. Of relevance to this review, the cAMP-elevating adenosine receptors were shown to be immunosuppressive in extensive pharmacological studies (Reviewed in [30]).

Currently, there is a large body of evidence that extracellular adenosine has multiple effects on immune cells. Briefly, adenosine signaling through A_{2A} suppresses functions of NK cells [37], NKT cells [38, 39], macrophages [40], dendritic cells [41] and granulocytes [42]. In T cells, stimulation of A_{2A} strongly inhibits proliferation, production of cytokines and induction of cytotoxic effectors [26, 43, 44]. T cell suppression by A_{2A} signaling is attributable to the inhibition of T cell receptor signaling. Downstream induction of cAMP and PKA activation interfere with intracellular Ca²⁺ increase and phosphorylation of ZAP70, Akt and ERK [45, 46]. A_{2A}-mediated signaling may downregulate TCR-signaling by PKA-mediated phosphorylation of COOH-terminal Src kinase (Csk) leading to inhibition of Lck activation [47].

A_{2A}-mediated inhibition of T cell effector functions

Adenosine-A_{2A} interaction causes general inhibition of T cell functions, but the extent of inhibition varies depending on specific function. Adenosine strongly impairs development of effector T cells, especially those that produce the proinflammatory cytokines IFN- γ or are cytotoxic to tumor cells. Conversely, cell proliferation of the same cells is relatively resistant to the inhibition by adenosine [43]. The selective inhibition of effector functions is persistent in activated T cells. Priming of T cells in the presence of A_{2A} agonist produced activated T cells incapable of producing high levels of IFN- γ even after the removal of A_{2A}

agonist. The A2AR-mediated induction of anergic T cells suggests long-lasting memory of exposure to the immunosuppressive effects of extracellular adenosine [27, 43, 48].

A2AR-mediated promotion of regulatory T (Treg) cell functions

The adenosine-A2AR pathway can suppress T cell immunity not only by direct action on effector T cells, but also indirectly by creating an immunosuppressive environment with massive Treg cell populations. A2AR stimulation was shown to upregulate FoxP3 mRNA [48] and immunoregulatory activity of CD4⁺ FoxP3⁺ cells [49]. In mixed-lymphocyte culture, A2AR agonists increased the number of CD4⁺ FoxP3⁺ cells with higher immunoregulatory activity, while activation of effector T cells was largely diminished [49]. This mechanism may be relevant to the documented and much discussed increase of Treg cells within tumors.

We proposed a model to potentially unify the diverse functions of Treg cells which is based on the assumption that Treg cells are complementary to the immunosuppressive physiological hypoxia-adenosinergic tissue-protecting mechanism [13]. According to our model it is the cAMP response element (CRE)- and hypoxia response element (HRE)-mediated transcription in Treg and effector cells that play an important role in Treg cell development and effector functions in inflamed and cancerous tissue microenvironments. The cAMP-elevating A2A and A2B adenosine receptors inhibit the T cell receptor (TCR)-triggered activities and hypoxia inducible transcription factor 1alpha (HIF-1alpha) - and HRE- and CRE-driven activities of Treg cells are required to achieve a maximal level of immune suppression [13].

Thus, extracellular adenosine in tumors can suppress anti-tumor T cell responses by A) direct inhibition of effector T cell activation, B) induction of functionally impaired effector T cells, and C) enhancement of the numbers and activities of Treg cells. The immunosuppressive concentration of extracellular adenosine may provide rapid (mechanism A) and long-lasting (B and C) immunoregulatory effects.

Intervention to inflammation by modulating the adenosine-A2AR pathway

The importance of targeting adenosine receptors in drug development [50] was recognized by experts in drug design [25, 51] thereby providing important tools for fruitful considerations of targeting A2AR to develop anti-inflammatory drugs by Linden, Okusa and collaborators [52]. It was shown that the A2AR activation protected from renal ischemia-reperfusion [53, 54] and reduced paralysis after spinal cord ischemia [55].

Some of pharmacological studies of adenosine receptors had important implications not only for drug development, but also for the understanding of the underlying immunological mechanisms in diseases. An appealing example of pharmacological research clarifying the role of the specific subtype of immune cells in pathophysiological processes was provided by the demonstrations that A2AR can reduce hepatic ischemia reperfusion injury by inhibiting CD1d-dependent NKT cell activation [38]. These observations pointed out the previously unappreciated role of NKT cells. It was also shown that dendritic cells-mediated NKT cell activation must be targeted to prevent the initiation of the immune response that causes kidney injury. Authors proposed to treat dendritic cells ex vivo with the A2AR-agonist in order to tolerize them, thus preventing dendritic cell-mediated activation of NKT cells in vivo and decreasing kidney injury [56].

In an important advance in understanding of the mechanisms of action of major drugs, elegant studies from Cronstein's group tested a non-obvious idea and demonstrated that A2AR and A3 adenosine receptor signaling could be the mechanism of action of the

methotrexate by pharmacologically stimulating the endogenous release of extracellular adenosine from connective tissues [57–60]. Yet another important finding was that ability of A2AR agonists to promote wound healing [61] and participate in fibrosis [62]. According to these studies, extracellular adenosine and A2 adenosine receptor signaling may represent the business end of the action of some drugs [57, 58].

Implications of demonstrations that extracellular adenosine may be generated from extracellular ATP

Generation of extracellular adenosine from extracellular adenosine 5'-monophosphate is shown to be mediated by ecto-5'-nucleotidase CD73 on the surface of some cell types. Using genetic controls, Linda Thompson as well as other investigators studied different models of diseases and implicated CD73 as a critical modulator of tissue protecting mechanisms [63]. Studies from Simon Robson's group established an appealing mechanism whereby the ectoenzyme CD39 acts upstream and in tandem with CD73 to generate adenosine from extracellular ATP and AMP [64–66].

CD73-mediated adenosine accumulation may be additive with the accumulation of extracellular adenosine from intracellular pools [67]. Based on studies of the AMP-adenosine metabolic cycle and the relation between AMP and adenosine formation as well as studies examining the effect of oxygen exposure (10%–95% O₂) on isolated guinea pig hearts [67], authors suggested that adenosine formation depends on the intracellular AMP concentration and that it is the hypoxia-mediated inhibition of adenosine kinase (AK) that results in the amplification of small changes in free AMP into a major rise in adenosine.

Such critical role of AK in modulating adenosine responses was further supported by observations of effects of hypoxia and of hypoxia inducible factor 1- α (HIF-1 α) in transcriptional repression of AK. Authors suggested the use of pharmacologic inhibitors of AK to treat hypoxia-induced vascular leakage during sepsis or acute lung injury [68, 69].

Motivational significance of the *in vivo* evidence for the critical role of A2AR in the protection of normal tissues during anti-pathogen immune response

There is high threshold in deciding to engage in projects focused on pharmacological treatments of cancer by immunoenhancement in which strong justification is required to support the molecular target of choice. This is why multiple correlative observations of the presence of different metabolites and proteins in TME were suggestive of their potential role in tumor biology and tumor immunology, but not sufficient to justify the choice of just one of many potential molecules as the subject of intensive, laborious and expensive *in vivo* and *in vitro* studies. This was the situation with considerations of spontaneously formed extracellular adenosine *in vivo* beyond its elegantly demonstrated role as the pharmacologically-induced immunosuppressor [57–60].

Indeed, in order to test which - if any- of G protein-coupled receptors (GPCR) are immunosuppressive *in vivo* in a specified microenvironment of inflamed or cancerous tissues, there is a need to know i) which specific type of immune cells are active in inflamed or cancerous tissue microenvironments; ii) whether there are sufficiently high levels of different endogenously formed GPCR ligands in the vicinity of immune cells in this microenvironment; iii) the repertoire of different GPCR on different subtypes of activated immune cells and iv) whether there are differences between inflamed tissue

microenvironments depending on the anatomical location of the inflammatory or cancerous tissues.

Some of the experiments to answer the questions poised above are not feasible, but one decisive experiment that would answer some of these questions would be to test the outcome of the genetic deletion of the suspected GPCR. If the GPCR is functioning as an constitutive immunosuppressor in inflamed tissues, then the deletion of this GPCR would cause the exacerbation of the immune response.

Accordingly, it was important to determine whether observations of immunosuppression *in vivo* due to the pharmacological activation of A2AR [52–60] could be reproduced *in vivo* without pharmacological introduction of the A2AR ligand. This was done by comparing intensities of immune responses in wild type mice and A2AR gene-deficient mice. It was expected that if the A2AR is indeed inhibiting overactive immune cells from inflicting excessive collateral damage, then in the absence of the tissue-protecting A2AR there would be more pro-inflammatory cytotoxic molecules and more collateral damage to normal tissues.

By employing different disease pathogenesis models, it was shown that the absence of A2AR did indeed enhance the immune response and exacerbated collateral damage to normal tissues [70–72]. The exacerbation of inflammatory tissue damage in A2AR-deficient mice occurred despite the fact that these mice still had all other cAMP-elevating receptors. The conclusion was that endogenous ligands of other GPCR could not compensate for the absence of A2AR in these assays, even though the pharmacological activation of other GPCR was capable of inhibiting the immune response in A2AR deficient mice [70]. It was also shown that A2AR inhibits Toll-like receptor-induced transcription of proinflammatory cytokines *in vivo*. The increase in mRNA levels of pro-inflammatory cytokines observed in A2AR-deficient mice was associated with enhanced activation of NF- κ B [71].

Taken together these observations provided the *in vivo* evidence for physiological attenuation of inflammatory processes by A2AR –and by extension-provided the genetic evidence for the immunosuppressive role of extracellular adenosine *in vivo*. These experiments had important implications for the understanding of the mechanisms of tumor protection since they established that A2AR is a critical and non-redundant negative regulator of immune cells in protecting normal tissues from inflammatory damage. Therefore, any tissue microenvironment, including the TME, will be immunosuppressive if the A2AR-expressing immune cells are exposed to extracellular adenosine.

The adenosinergic pathway in the protection of cancerous tissues from tumor- reactive T cells

The demonstration that A2AR is the physiological inhibitor of immune cells, including T cells, suggested that the inhibition of incoming anti-tumor T cells in TME could be mediated by tumor-produced extracellular adenosine. To test whether A2AR on T cells protects tumors from anti-tumor effector cells, tumor growth was evaluated in wild type mice and A2AR-deficient mice or in wild type mice treated with A2AR antagonists [73].

It was shown that the genetic deletion of A2AR resulted in a rejection of established immunogenic tumors in a majority of A2AR-deficient mice with no rejection of tumors in control wild type mice. The anti-tumor effects of deleting A2AR signaling were dependent on CD8⁺ and CD4⁺ T cells since knock-down of the A2 receptors by siRNA in tumor-reactive T cells prior to adoptive transfer into tumor-bearing mice improved the inhibition of tumor growth. These data suggest that the effects of A2AR are T cell autonomous [73].

The anti-tumor effects of genetic deletion of A2AR, including the inhibition of tumor growth, destruction of metastases, and the prevention of neovascularization in tumors, have been reproduced using selective synthetic A2AR antagonists and the natural adenosine receptor antagonist, caffeine [73]. However, pharmacological A2AR antagonism of the tested drugs had much weaker anti-tumor effects than genetic deletion of A2AR and no survival was accomplished with tested antagonists. Enhanced rejection of transplantable tumors by endogenously developed anti-tumor T cells with genetic deficiency of A2AR suggests that the reason for the weaker effects of antagonists could be due to the quality of these drugs, e.g. short half-life *in vivo*. This in turn offers the hope that using longer-lived and more selective synthetic A2AR antagonists may accomplish better tumor rejection by cancer vaccine-induced endogenous T cells.

The A2AR-mediated inhibition of anti-tumor T cells in the adenosine-rich TME may explain the paradoxical coexistence of tumors and antitumor immune cells in some cancer patients (the “Hellstrom paradox”). This, in turn, led to a proposal to weaken the immunosuppressive extracellular adenosine-A2AR pathway in the TME as a novel strategy for cancer immunotherapy [73–75]. The same strategy may prevent the premature termination of immune response and improve the vaccine-induced development of anti-tumor and anti-viral T cells. The observations of autoimmunity during melanoma rejection in A2AR-deficient mice suggested that the A2AR on T cells is also important in preventing autoimmunity. Thus, although inhibition of the extracellular adenosine-A2AR pathway may improve anti-tumor immunity, the recruitment of this pathway by selective drugs is expected to attenuate autoimmune tissue damage. The critical role of the extracellular adenosine-A2AR immunosuppression in the TME was confirmed and extended by others in a series of mouse genetic and pharmacological experiments [76–82].

Taken together these data provided the first conclusive evidence for the immunosuppressive role of extracellular adenosine and of A2AR on T cells in the protection of tumors in the TME. This conclusion is supported by the earlier important observations of increased concentrations of extracellular adenosine in the TME [83, 84]. However, the general limitations of the pharmacologic approach did not allow for correct identification of the physiologically activated A2AR in those studies and, instead, pointed to A3 adenosine receptor [85] or to the “novel non-A1/A2” adenosine cell-surface receptor [86] as inhibitory signal-transducing receptors that prevent induction of anti-CD3-activated killer T cells.

Recently, another Gs protein-coupled adenosine receptor, A2BR, was also found to play a significant role in the modulation of inflammation. Compared to A2AR, A2BR is a low-affinity receptor and requires higher concentration of adenosine. However, in hypoxic environments, local adenosine concentration can increase to levels sufficient to activate A2BR [87]. Hypoxia was found to upregulate A2BR levels in a HIF-1 α -dependent manner [88–90]. A2BR stimulation may also promote its immunosuppressive effect by increasing local numbers of Treg cells [91] and myeloid-derived suppressor cells [92]. These findings suggest that, in addition to A2AR, A2BR is involved in the establishment of an immunosuppressive TME. This speculation is consistent with the observation of tumor growth retardation in A2BR-deficient mice [93] and in wild-type mice treated with A2BR antagonist [94].

Preparation of tumor-reactive T cells, which are less inhibitable by adenosine in TME

The elimination or antagonism of A2AR on anti-tumor T cells is an attractive novel cancer immunotherapy approach, but an additional useful methodology would be to decrease the number of A2AR per T cell [73–75]. This suggestion is based on observations of the

absence of A2AR “reserve” on T cells [29]. Therefore, decreasing the level of A2AR on the surface of anti-tumor T cells would decrease the susceptibility of T cells to inhibition by the tumor-produced adenosine.

We attempted to develop a method of decreasing the expression of A2AR and A2BR on anti-tumor T cells during their expansion *in vitro* for subsequent adoptive transfer. The idea was to remove those T cells that have high levels of A2AR and A2BR. When T cells were cultured in the presence of A2AR agonist, it is assumed that adenosine-prone T cells will have a disadvantage in terms of activation while T cells that manage to proliferate in such conditions are expected to be more resistant to inhibition by extracellular adenosine.

In these experiments, cytotoxic T lymphocytes (CTL) were developed in the presence of an adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA). As expected, the CTL which were capable of proliferating even in the presence of NECA were less susceptible to inhibition by A2AR agonists, as shown by i) a much smaller accumulation of cAMP and ii) lesser inhibition of IFN- γ production compared with control CTL. Importantly, the production of CTL that are both resistant to adenosine-mediated immunosuppression and maintain strong cytotoxicity and IFN- γ secretion required the presence of NECA only during the expansion stage after the establishment of CTL. In contrast, the priming of resting T cells in the presence of NECA resulted in T cells with impaired effector functions. These *in vitro* observations suggested the improvement of current protocols to produce anti-tumour T cells that are more effective in adoptive immunotherapy of cancer [95].

It is important to clarify that this procedure is not likely to be selection of A2AR^{low} cells because the NECA-treated cells regained normal A2AR responsiveness and sensitivity after a couple of days. A temporary surface A2AR down-regulation is more likely.

Conclusion

Thus, although the adenosine-rich environment in the TME may allow for the expansion of T cells, the functional activation of T cells may be critically impaired. This physiological mechanism could explain the inefficiency of anti-tumor T cells in the TME. [43]. The expansion of such functionally impaired T cells amid adenosine may be relevant to unresponsiveness of anti-tumor effectors in the TME. Tumor-infiltrating T cells are capable of recognizing tumor-associated antigens. The presence of these anti-tumor effectors in tumors indicates that T cell priming to tumor-associated antigens and mobilization of these T cells have occurred. Although tumor-infiltrating T cells keep the potential to destroy tumor cells, their effector functions are critically disabled in the TME [96, 97]. Enhanced tumor eradication by T cells lacking A2AR suggests the involvement of intratumoral adenosine in the down-regulation of T cell effector functions.

The studies described above on hypoxia–adenosinergic immunosuppression in the TME may have accelerated clinical implications since the selective and long-lived *in vivo* A2AR antagonists have been developed for treatment of Parkinson's disease and have been shown to be safe and well tolerated [25, 98]. The added advantage is that the anti-A2A-adenosinergic drugs are expected to, at least partially, prevent inhibition of anti-tumor immunity in the TME by other immunosuppressive pathways, such as FoxP3⁺ Treg cells [13, 99–102] cyclooxygenase-2 and the production of PGE₂ [103, 104] thereby further enhancing anti-tumor immunity.

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Figure 1.

Improvement of anti-tumor immune responses by preventing immunosuppressive effects from the adenosine-A2AR pathway. Extracellular levels of adenosine in tumors are higher than in normal tissues due, at least in part, to hypoxia. Extracellular adenosine binds to A2AR and/or A2BR on the surface of anti-tumor effector cells and subsequently increases cAMP levels. To break this potentially immunosuppressive sequence, therapeutic targets will include the accumulation of extracellular adenosine, binding of adenosine to A2AR/A2BR and cellular expression of A2AR/A2BR. Inhibitor of ectonucleotidases (e.g. CD73) can diminish metabolic production of adenosine from adenosine phosphates. Antagonists of A2AR/A2BR can block interaction of adenosine with the cell surface receptors. In adoptive immunotherapy, anti-tumor effector cells may be pretreated to be insensitive to adenosine by elimination of the receptor expression with siRNA or by simply culturing the cells with adenosine receptor agonist. Such treatment is expected to abolish adenosine-mediated immunosuppression in tumor microenvironment and promote tumor eradication by immune cells.