18 Adenosine, Tumors, and Immunity

Pnina Fishman, Sara Bar-Yehuda, and Lea Madi

CONTENTS

18.1 INTRODUCTION

Considerable evidence has accumulated indicating that adenosine plays an important role in controlling tumorigenesis via its receptors. Adenosine, which is released to the microenvironment by metabolically active tumor cells, fulfils a multitude of functions in regulating tumor cell proliferation. At micromolar (μM) concentrations

it directly induces an antiproliferative effect toward various tumor cell types. Indirectly, it affects tumor development via its capability to affect cytokine release, cell migration, angiogenesis, and chemotaxis. Moreover, adenosine induces activation or suppression of T killer or natural killer cells that affect tumor cell development.

It is quite impossible to assess the effect of adenosine *in vivo* because of its rapid metabolization by adenosine deaminase (ADA). However, its effects are mediated via the four receptor subclasses: A_1, A_{2A}, A_{2B}, and A_3.

This chapter briefly reviews the role played by various adenosine receptors in mediating tumor cell response to adenosine, its agonists, and antagonists.

Interestingly, among the four receptor subtypes, the A_3AR was found to mediate a potent antitumor effect. The specificity of this target results from the finding that this receptor is highly expressed in tumor cells, whereas low receptor expression is reported in normal cells. In addition, the low affinity of adenosine to A_3AR and the finding that A_3AR knockout mice are considered normal indicate that the receptor does not mediate essential functions under normal conditions, and thus may be suggested as a specific target to combat cancerous diseases. The associated molecular mechanisms, including signal transduction pathways generated upon adenosine receptor activation in tumor cells, will be described in this chapter.

18.2   THE EFFECT OF ADENOSINE ON TUMOR CELL GROWTH

Rapidly growing solid tumor tissues are characterized by the high metabolic rate of the proliferating tumor cells and frequently insufficient microcirculation. As a result, local regions of ischemia and hypoxia are developed.[1] Under these conditions adenosine levels are elevated owing to three pathways that involve breakdown of ATP to AMP: via the 5'-nucleotidase, decreased AMP formation by the inhibition of adenosine kinase, and upregulation of *S*-adenosylhomocysteine hydrolase, which also participates in adenosine formation.[2–4] The concentration of adenosine in extracellular fluids of colorectal and lung carcinoma tumor tissues can reach up to 10^5 mol/l.[5]

The high adenosine level in the tumor microenvironment prompted the study of adenosine's effect on leukemia, lymphoma, and solid tumor cell types. The antitumor effect of adenosine, mediated via mechanisms dependent or independent of adenosine receptors, results in cell cycle arrest, proliferation inhibition, or the induction of apoptosis.[6–10]

In the human leukemia cell lines HL-60 and K-562 as well as in the Nb2-11C rat lymphoma cells, adenosine at *μM* concentrations causes inhibition of tumor cell growth. In some cell types the inhibitory effect was due to apoptosis induced via an active transport of adenosine into the cells.[8,11]. In other cell types adenosine induced a cytostatic effect by arresting the cells in the G_0/G_1 phase of the cell cycle as a result of a decrease in the telomeric signal. Telomeres are repeated DNA sequences that guard the ends of chromosomes, serving as a checkpoint for cell-cycle progression, thus regulating cell senescence and apoptosis.[12]

Few studies have tested the response of solid tumors to adenosine. Low concentrations of adenosine ($<10\,\mu M$) induced cell growth inhibition in A431 human epidermoid carcinoma cells, LNCaP human prostate adenocarcinoma, and murine B16-F10 melanoma. At higher concentrations, adenosine promoted cell proliferation of the

A431 human epidermoid carcinoma cells through activation of the A_2 adenosine receptors.[8,13]

Adenosine was shown to induce a differential effect on tumor and normal cell growth. The proliferation of lymphocytes derived from patients with chronic lymphocytic leukemia was suppressed by adenosine, whereas that of normal lymphocytes was inhibited to a lesser extent.[14] Moreover, stimulation of normal cell proliferation by adenosine has been demonstrated in Swiss mouse 3T3 and 3T6 fibroblasts, thymocytes, hemopoietic cells, endothelial cells, astrocytes, and myeloid bone marrow cells.[8,15–18]

18.2.1 THE ANTITUMOR EFFECT OF ADENOSINE: MEDIATED VIA ADENOSINE RECEPTORS

The effect of adenosine and its agonists/antagonists on tumor cells depends on their extracellular concentrations and on the expression of different adenosine receptor subtypes. Upon receptor activation, various signal transduction pathways are generated, resulting in a direct inhibitory effect on tumor growth. Other cell types, such as immunocytes or endothelial cells, may respond to receptor activation by the release of cytokines and mediators that indirectly affect tumor growth.

18.2.1.1 Tumor Cells and Expression of A_1 and A_3 Adenosine Receptors

Studies aimed at the analysis of adenosine receptor expression in tumor cells were conducted based on previous knowledge that high adenosine level in the tumor microenviroment may regulate receptor expression and that receptor density may affect cell response to adenosine or its synthetic agonist/antagonists. This led to studies exploring high receptor expression as a characteristic of tumor cells. There is evidence that the A_1 adenosine receptor is expressed in various tumor cell lines, including the A431 epidermal carcinoma, LOVO human colon carcinoma, A2058 melanoma, medullary thyroid carcinoma, and human hepatocellular carcinoma cells.[19–22] In human colorectal cancer tissue the mRNA expression level of A_1AR was found to be high compared to the peritumoral tissue, whereas A_{2A}A expression level was the same in both tissues.[23]

A_3AR expression was also found in tumor cell lines including astrocytoma, HL-60 leukemia, B16-F10 and A378 melanoma, human Jurkat T cell lymphoma, and murine pineal tumor cell.[24–30]

High A_3AR mRNA, protein expression level, and cell surface exhibition were reported in different tumor cell types. A retrospective study in pathological paraffin embedded slides of breast, colon, pancreatic, small cell lung carcinoma, and melanoma showed high A_3AR expression in comparison to normal adjacent tissues. Protein A_3AR expression level was high in fresh tumors derived from colon and breast carcinoma when compared to normal adjacent tissue, as detected by Western blot (WB), immunohistochemistry, and binding assay analyses.[29,31]

Gradual increase in A_3AR density is seen during colorectal tumor progression, and is manifested by greater receptor density as the tumor develops from a small adenoma into a large adenoma and finally to colon carcinoma. Moreover, mRNA A_3AR expression level was higher in lymph node metastasis compared to primary

tumor and normal peritumoral tissue. Interestingly, in peripheral blood lymphocytes and neutrophils of colon carcinoma patients, high A_3AR density was detected and reflected A_3AR expression in the relevant tumor tissue.[29,31]

It thus seems that A_3AR levels are directly correlated to tumorigenicity and may be developed as an additional biomarker to monitor disease progression.

18.2.2   DIRECT ANTITUMOR EFFECTS INDUCED BY A_3AR AGONISTS AND MECHANISMS INVOLVED

Although various tumor cells highly express A_1 and A_3 adenosine receptors, the anticancer activity is attributed to the A_3AR. Growth inhibition of tumor cells upon A_3AR agonist treatment was reported to be mediated via cell cycle arrest, apoptosis, or necrosis, depending on cell type and agonist concentration. Moreover, inhibition of tumor growth by A_3AR synthetic agonists was found to be receptor dependent or receptor independent.

Tumor and normal cells respond differentially to the activation of A_3AR by natural or synthetic agonists. Utilizing nanomolar (n*M*) A_3AR agonist concentrations, inhibition of tumor cell growth *in vitro* was observed in melanoma, colon, breast, and prostate carcinoma.[32–36] On the other hand, the proliferation of normal cells such as murine bone marrow was stimulated.[37,38] At *µM* concentrations, the proliferation of both tumor and normal cells was inhibited. This differential effect may be explained by the high vs. low A_3AR expression level in tumor and normal cells, respectively.

18.2.2.1   Antitumor Effect of A_3AR Agonists at *µM* Concentrations

At *µM* concentrations, A_3AR agonists inhibited the growth of leukemia, lymphoma, and various solid tumor cell lines.

In HL-60 promyelocytic leukemia and U-937 hitiocytic lymphoma cells, A_3AR agonists, 1-deoxy-1-amino]-9H-purine-9-yl]-*N*-methyl-(-D-ribofuranuronamide (IB-MECA), and 2-chloro-*N*[6]-(3-iodobenzyl)-adenosine-5-*N*-methyluronamide (CL-IB-MECA) induced apoptosis via an elevation in intracellular calcium concentration and upregulation of the Bak gene. However, low concentrations of these agonists protected against apoptosis induced by A_3AR antagonists.[39,40] In an additional study that also included the HL-60 cells and MOLT-4 cells, apoptosis was induced by Cl-IB-MECA and was not mediated via the A_3AR. The mechanism proposed included upregulation of the death receptor, Fas, which was p53 independent.[41]

In solid tumors, the effect of IB-MECA at *µM* concentrations was tested in different breast cancer cell lines including MCF-7, ZR-75 and T47D (estrogen receptor α positive) MDA-MB468, and Hs578T (estrogen receptor α negative). Panjehpour et al. (42) reported that in the MCF-7 and the MDA-MB468 cell lines, IB-MECA treatment resulted in significant cell growth inhibition. The response was A_3AR mediated, as was demonstrated by pretreatment with the selective A_3AR antagonist MRS1220 and by the inhibition of forskolin-stimulated cAMP levels.[42] In another study Lu et al.[43] also demonstrated that IB-MECA induced growth inhibition of MCF-7 and ZR-75 and apoptosis in T47D and

Hs578T cells. The authors showed that the response to IB-MECA was not A_3AR mediated (because they did not detect A_3AR mRNA expression in these cells) and that the mechanism involved downregulation of the mRNA and protein expression level of the estrogen receptor.[43]

In melanoma tumor cells the A_3AR agonist Cl-IB-MECA inhibited the proliferation of the A375 melanoma cell line via an A_3AR dependent pathway. This was supported by studies showing that the response to A_3AR was neutralized by a specific antagonist and by A_3AR knock-down (using the siRNA technique). The mechanism of action included modulation of the phosphatidylinositide-3-OH kinase (PI3K)/Akt and the Raf/mitogen-activated protein kinase, MAPK/Erk kinase, and MEK/mitogen-activated protein kinase (MAPK) pathways. Stimulation of the PI3K-dependent phosphorylation of Akt leading to the reduction of basal levels of ERK1/2 phosphorylation levels took place.[44]

In an additional set of experiments IB-MECA and Cl-IB-MECA at a concentration of 10 μM inhibited the proliferation of murine melanoma (B16-F10), human colon (HCT-116), and prostate carcinoma (PC-3) cells. The inhibitory effect was only partially mediated via the A_3AR because the A_3AR antagonist MRS 1523 neutralized only 50% of the agonist inhibitory effect.[34,35]

18.2.2.2 Antitumor Effect of A_3AR Agonists at nM Concentrations

In contrast to adenosine, which binds to all the different receptor subclasses, A_3AR synthetic agonists, which have high affinity to the receptor, will activate A_3AR exclusively. This was the rationale behind the examination of the effect of IB-MECA and Cl-IB-MECA (having affinity values in the range of 0.5 to 1.5 nM) on the growth of different tumor cells at nM concentrations.

IB-MECA and Cl-IB-MECA inhibited the proliferation of murine melanoma (B16-F10), human colon (HCT-116), and prostate carcinoma (PC-3) in a dose-dependent manner. Cell proliferation was measured by [^{3}H]-thymidine incorporation at nanomolar concentrations (1 to 1000 nM). The response was A_3AR dependent, because the effect was fully neutralized by the antagonist MRS 1523, suggesting that at low nM concentrations, only A_3AR is activated, mediating the antitumor effect.[33–35]

Cross talk between the A_3AR and the Wnt signal transduction pathway was found to play a major role in mediating the inhibitory effect of IB-MECA (at 10 nM concentration) on the growth of melanoma, colon, and prostate carcinoma. Downregulation of receptor expression was noted shortly after A_3AR activation, in the three cell lines, demonstrating the rapid response of the tumor cells to agonist stimulation and the initiation of downstream responses. Indeed, the tumor cells responded to A_3AR activation by a decrease in protein kinase A (PKA) c level, an effector protein involved in the initiation, regulation and cross talk between various signaling pathways. It phosphorylates and inactivates the enzyme glycogen synthase kinase 3 (GSK-3β), a key element in the Wnt signaling pathway.[45,46] GSK-3β, suppresses mammalian cell proliferation and survival by phosphorylating the cytoplasmic protein β-catenin, leading to its ubiquitination. GSK-3β in its inactive form does not phosphorylate β-catenin, which accumulates in the cytoplasm and subsequently translocates to the nucleus, where it associates with Lef/Tcf to induce cyclin-D1 and c-myc transcription.[47] IB-MECA treatment decreased the PKA and protein

kinase B/Akt (PKB/Akt) levels. Subsequently, the phosphorylated form of GSK-3β was decreased and total GSK-3β levels were increased. This led to downregulation in the level of β-catenin, Lef/Tcf, and the β-catenin-responsive cell growth regulatory genes c-myc and cyclin D1. These observations, which link cAMP to the Wnt signaling pathway, provide mechanistic evidence for the involvement of the Wnt pathway, via its key elements GSK-3β and β-catenin, in the antitumor activity of A₃AR agonists (See Figure 18.1)

The expression level of NF-κB was downregulated in both *in vitro* and *in vivo* studies. NF-κB is also linked to the effector protein PKAc. The most abundant form of NF-κB is a heterodimer of p50 and p65 (Rel A) subunits in which the p65 contains the transcription activation domain. PKAc regulates the transcriptional activity of NF-κB by phosphorylating the p65 subunit of NF-κB, enabling its association with the coactivator CBP/p300 and efficient transcriptional activity.[48,49] Thus, IB-MECA's capability to suppress NF-κB expression may serve as part of the mechanism through which it exerts an inhibitory effect on tumor growth (See Figure 18.1).
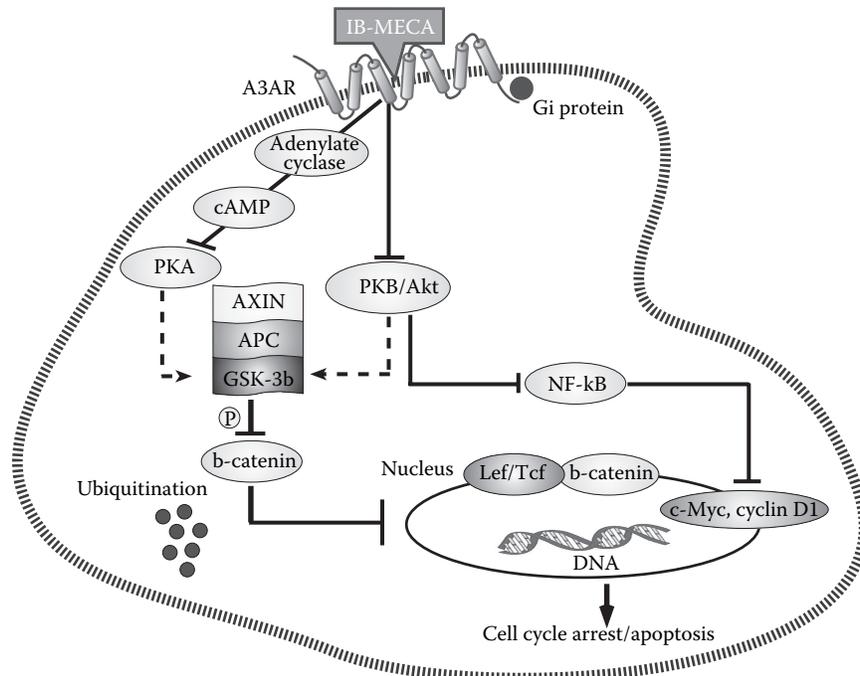
FIGURE 18.1 Activation of A₃AR by IB-MECA deregulates the Wnt and the NF-κB signaling pathways. Tumor cells responded to A₃AR activation by a decrease in the levels of PKAc and PKB/Akt. Both are known to control the phosphorylation and inactivation of GSK-3β. PKB/Akt also phosphorylates downstream proteins such as IKK and I B. As a result the expression level and activity of -catenin, Lef/TCF, and NF-κB is downregulated. This chain of events is followed by decreased transcription of cell growth regulatory genes, such as c-myc and cyclin D1, resulting in cell cycle arrest and apoptosis.

18.2.2.3 *In vivo* Effect of A$_3$AR Agonists

Oral administration of IB-MECA and Cl-IB-MECA was efficacious in inhibiting the development of primary tumors and metastasis in xenograft and syngeneic models. IB-MECA inhibited the development of primary colon carcinoma in syngeneic (murine CT-26 colon carcinoma and B16-F10 melanoma cells) and xenograft (HCT-116 colon and PC3 prostate human carcinoma cells) tumor models. Moreover, IB-MECA and Cl-IB-MECA suppressed the development of lung melanoma metastases and IB-MECA inhibited colon cancer liver metastases in syngeneic mice. In all *in vivo* experiments, mice were treated with a low dose of agonist (10 µg/kg) aimed at exclusively targeting the A$_3$AR.[32–38]

Exploration of the mechanism responsible for the *in vivo* antitumor effect was carried out in protein extracts derived from IB-MECA-treated melanoma prostate and colon–carcinoma-bearing animals. A similar protein profile to that described *in vitro* was found, suggesting that the Wnt and the NF-κB signal transduction pathways are involved in the *in vivo* antitumor effect.[32–38] Table 18.1 summarizes the different experimental animal models used and the percentage of tumor growth inhibition exerted by the two A$_3$AR agonists.

18.2.2.4 A$_3$AR Fate upon Chronic Activation

It is well established that upon activation of a G$_i$ protein receptor, a chain of events take place including phosphorylation, palmitoylation, desensitization, internalization, receptor downregulation, resynthesis, and recycling as a functional receptor to

TABLE 18.1
**Effect of A$_3$AR Agonists on Tumor Growth**

Percentage of Tumor Growth Inhibition (at Study Termination)	Tumor Type	Experimental Model
52 ± 2.8	B16-F10, murine melanoma	Primary
53 ± 4.7	CT-26, murine colon carcinoma	Primary
63 ± 8.2	HCT-116, human colon carcinoma	Primary
75 ± 9.7	PC3, human prostate carcinoma	Primary
59 ± 6.8	B16-F10, murine melanoma Lung metastases	Metastatic
52 ± 8.3	CT-26, murine colon carcinoma Liver metastases	Metastatic

Note: In vivo, oral administration of A$_3$AR agonists was efficacious in inhibiting the development of primary tumors and metastasis in xenograft and syngeneic models. IB-MECA inhibited the development of primary colon carcinoma in syngeneic (murine CT-26 colon carcinoma and B16-F10 melanoma cells and xenograft tumor models (HCT-116 colon and PC3 prostate human carcinoma cells). Moreover, IB-MECA and Cl-IB-MECA suppressed the development of lung melanoma metastases, and IB-MECA inhibited colon cancer liver metastases in syngeneic mice.

AU: Closing parenenthesis missing. Please add.

the cell surface (see Figure 18.2). This process leads to the initiation of a downstream molecular mechanism that transmits the signal to the nucleus (Figure 18.2). As was specified earlier, the A_3AR expression level is highly expressed in various leukemias, lymphomas, and solid tumors. Given that A_3AR agonists inhibit the growth of cells, receptor fate upon chronic activation and the effects on the downstream molecular mechanisms leading to tumor growth inhibition, both *in vitro* and *in vivo*, are important. When the agonist is used as a drug, chronic targeting of the Gi protein receptor might lead to its desensitization and loss of function, resulting in a lack of response.

Only two studies were conducted that investigated receptor fate in tumor cells. In the ADF astrocytoma cell line, A_3AR fate was examined after short- and long-term agonist exposure. A_3AR desensitization, tested by the adenylyl cyclase activity assay, occurred shortly (15 min) after activation with the agonist Cl-IB-MECA (100 n*M*).
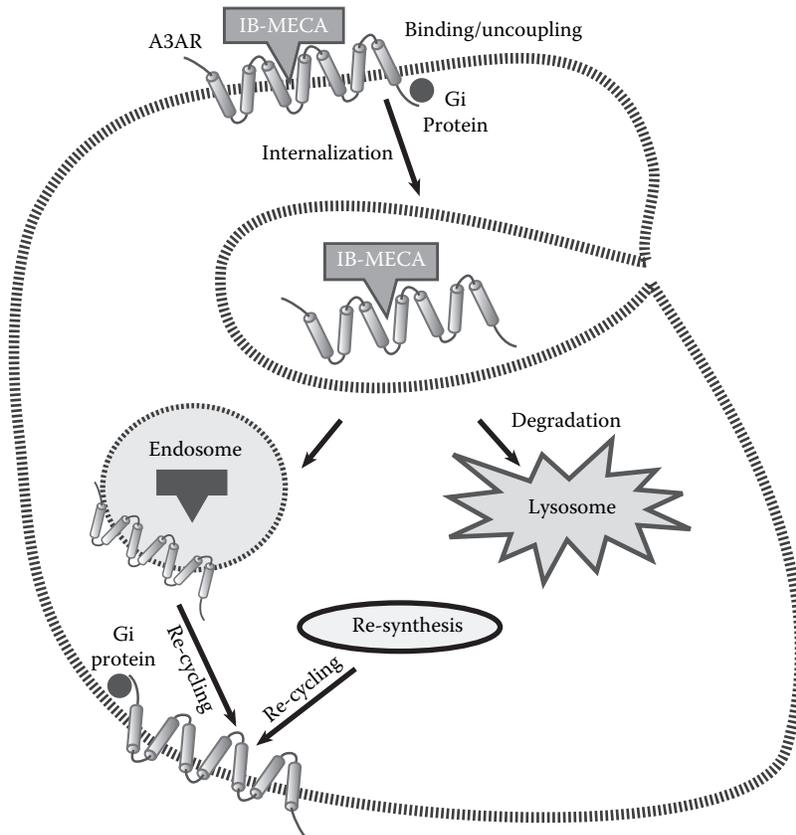
FIGURE 18.2 Upon activation of A_3AR by IB-MECA, desensitization and internalization of the receptor take place. Within the cells the receptor accumulates either in the endosome to be recycled to the cell surface or in the lysosome for degradation. In parallel, resynthesis of a functional receptor that will also be recycled to cell surface may occur.

Desensitization was accompanied by receptor internalization (30 min) and intracellular distribution to various compartments. Recycling and restoration of receptor function were observed upon agonist removal (120 min). The receptor was tracked by utilizing radioligand binding studies and by immunogold staining, followed by electron microscopy examination. Downregulation of the receptor, as analyzed by Western blotting analysis, took place after prolonged agonist exposure (1 to 24 h). Restoration of cell surface receptor to control values was followed by recovery of receptor functioning.[28]

A$_3$AR fate was also studied in B16-F10 melanoma cells, in which the receptor was highly expressed, and was tracked by confocal microscopy and radioligand binding. Upon chronic *in vitro* exposure to IB-MECA, gradual internalization occurred within a few minutes, whereas after 15 min receptor recycling to the cell surface was noted. Colocalization with FITC-dextran revealed that after internalization, the receptor was "sorted" to the early endosomes and recycled to the cell surface. In parallel, colocalization with FITC-transferrin showed that the receptor was targeted to lysosomes and then subjected to degradation. Receptor distribution in the lysosomes was consistent with the downregulation of receptor protein expression level. This was followed by mRNA and protein resynthesis and full receptor recovery (protein expression and cell surface exhibition) after 24 h. At each stage, receptor functionality was evidenced by the modulation in cAMP level and the downstream effectors PKA, GSK-3β, c-Myc, and cyclin D1.[36] Supporting the notion that the receptor is fully recovered upon chronic treatment are studies in experimental animal models of murine melanoma, as well as human prostate and colon carcinoma. Daily treatment with IB-MECA for one month resulted in receptor downregulation in tumor lesions shortly after treatment and full receptor recovery before the next treatment. The data showing receptor recovery after chronic activation *in vitro* and *in vivo* may suggest A$_3$AR as a stable target to combat tumor cell growth.[35–37]

18.2.3 INDIRECT ANTITUMOR EFFECTS MEDIATED VIA ADENOSINE AND ITS RECEPTORS

The high adenosine concentration in the tumor microenvironment raises the question of whether this natural ligand supports or inhibits tumor growth by activating or inhibiting immune cells and cytokines that play an important role in maintaining tumor development.

In a series of experiments, it was shown that adenosine prevented the induction of murine anti-CD-3-activated killer cells via A$_3$AR activation and suppressed β integrin-mediated adhesion of T lymphocytes to colon adenocarcinoma cells. In addition, high levels of adenosine or its agonists inhibited IL-12 and TNF-α production via activation of A$_2$ and A$_3$ adenosine receptors. IL-12 and TNF-α are known to act as anticancer cytokines and their inhibition supports tumor growth.[50–53]

On the other hand, the Cl-IB-MECA, at a low dose (10 μg/kg), potentiated the activity of NK cells in naïve and tumor-bearing mice. Cl-IB-MECA induced increased serum IL-12 levels followed by elevated NK cell activity and tumor growth inhibition.[54]

18.3 ENHANCEMENT OF CHEMOTHERAPEUTIC AND CHEMOPROTECTIVE EFFECTS VIA ADENOSINE RECEPTOR ACTIVATION

Adenosine's characteristic of inducing a differential effect on tumor and normal cells is mediated via the A_3AR and was the basis of examining the effect of A_3AR agonists as compounds that enhance the chemotherapeutic index and of testing their myelo-protective effects *in vivo*.

18.3.1 A_3AR AGONISTS AND ANTAGONISTS AND CHEMOTHERAPEUTIC INDEX ENHANCEMENT

NF-κB and the upstream kinase PKB/Akt are highly expressed in chemoresistant tumor cells and may hamper the apoptotic pathway. A_3AR agonists have been shown to down-regulate PKB/Akt and NF-κB protein expression level, a result that prompted the evaluation of their effect on the response of tumor cells to cytotoxic drugs. Combined treatment of IB-MECA and 5-Flurouracil (5-FU) enhanced the cytotoxic effect of the latter on HCT-116 human colon carcinoma growth. Downregulation of PKB/Akt, NF-κB, and cyclin D1 and upregulation of caspase-3 protein expression level was observed in cells and tumor lesions upon treatment with a combination of IB-MECA and 5-FU.[55]

In three different experimental murine models, including syngeneic (B16-F10 melanoma in C57Bl/6J mice) and xenograft models (HCT-116 human colon carcinoma and PC-3 human prostate carcinoma in nude mice), IB-MECA or Cl-IB-MECA inhibited tumor growth when administered orally at low doses (5 to 100 mg/kg). The tumor inhibitory effect was of the same magnitude as that seen with a standard chemotherapy protocol. When combined with chemotherapy, a synergistic effect was seen, yielding an overall, greater response than treatment with the agonists or chemotherapy alone.[56] The protein profile in tumor lesions excised from colon-carcinoma-bearing mice treated with a combination of 5-FU and IB-MECA revealed downregulation of PKB/Akt, NF-κB, and cyclin D1 and upregulation of caspase-3 protein expression level.[55] These data suggest that IB-MECA opens the way for apoptosis to take place via the modulation of apoptotic proteins.

A_3AR antagonists, pyrazolotriazolopyrimidine derivatives (PTP-d), were also shown to sensitize the A375 human melanoma cells to the chemotherapeutic agents taxol and vindesine. PTP-d's reduced the EC50 doses of the G_2/M accumulation by an average of 1.7-fold for taxol and 9.5-fold for vindesine and increased the ability of the melanoma cells to undergo apoptosis. PTP-d's were found to interact with a binding site on multidrug-resistance-associated ATP binding cassette drug transporter and to control ATP hydrolysis produced during the drug transport, thus affecting the activity of the chemotherapeutic agent.[57]

18.3.2 THE A_1 AND THE A_3 ADENOSINE RECEPTORS: MEDIATING CHEMOPROTECTIVE EFFECTS

Adenosine stimulates the proliferation of murine bone marrow cells *in vitro*. Pharmacological studies, using antagonists to the adenosine receptors, revealed that this activity was mediated through the binding of adenosine to its A_1 and A_3 receptors.

The two selective A_1 and A_3 receptor agonists, CPA and IB-MECA, induced bone marrow cell proliferation in a manner similar to adenosine.[58]

Adenosine's interaction with its A_1 and A_3 receptors induced G-CSF production *in vitro*, which led to its stimulatory effect on bone marrow cells. *In vivo* administration of low-dose adenosine (0.25 mg/kg) to mice pretreated with chemotherapy restored the number of leukocytes and neutrophils to normal levels, compared to the decline in these parameters after chemotherapy alone.[58]

Synthetic A_3AR agonists exhibited a myelostimulatory effect both *in vitro* and *in vivo*. IB-MECA and Cl-IB-MECA show an overall myeloprotective activity in mice pretreated with chemotherapy. Oral administration of IB-MECA to naive mice led to the elevation of serum G-CSF levels, an increase in absolute neutrophil counts (ANC), and bone marrow colony-forming cells. Splenocytes derived from these mice produced higher G-CSF levels than controls. The molecular mechanisms underlying the events prior to G-CSF production included the upregulation of NF-κB and the upstream PI3K, PKB/Akt, and IKK. Accelerated recovery of white blood cells and neutrophil counts were observed in cyclophosphamide-treated mice following IB-MECA administration. Thus, the NF-κB signaling pathway also plays a key role in mediating the myeloprotective effect of IB-MECA. In contrast to tumor cells, normal G-CSF-producing cells respond to IB-MECA by elevating NF-κB levels.[38]

In addition to the myeloprotective effect, A_3AR agonists were found to mediate a protective effect against doxorubicin-induced cardiotoxicity. Activation of A_3AR, but not A_1AR, attenuated doxorubicin cardiotoxicity in cultured cardiomyocytes. Protection by Cl-IB-MECA was manifested by a decrease in intracellular calcium, reduction of free-radical generation and lipid peroxidation, attenuation of mitochondrial damage, and attenuation of the decrease in ATP production. Cardioprotection caused by Cl-IB-MECA was antagonized considerably by the selective A_3AR antagonist, MRS1523, demonstrating the role of A_3AR in mediating the cardioprotective effect.[59]

18.4 CONCLUSIONS

The direct inhibitory effect of adenosine on tumor cell growth is mainly mediated via the A_3AR. Synthetic agonists to this receptor demonstrate an anticancer effect that is mediated via cell cycle arrest, apoptosis, or necrosis, dependent on agonist concentration and cell type. Molecular mechanisms involving modulation of the MAP kinase, Wnt, and the NF-κB signal transduction pathways are involved. The oral bioavailability of synthetic A_3AR agonists, and their induced systemic anticancer and myeloprotective effects, render them potentially useful in three different modes of treatment: as a stand-alone anticancer treatment, in combination with chemotherapy to enhance its therapeutic index, and for myeloprotection. A_3AR agonists are thus a promising new class of agents for cancer therapy.

REFERENCES

1. Vaupel, P., Kallinowski, F., and Okunieff, P., Blood flow, oxygen and nutrient supply, and metabolic microenvironment in solid tumors: a review, *Cancer Res.,* 51, 5139, 1989.
2. Headrick, J.P. and Willis, R.J., 5-Nucleotidase activity and adenosine formation in stimulated, hypoxic and underperfused rat heart, *Biochem. J.*, 261, 541, 1989.

3. Decking, U.K. et al., Hypoxia-induced inhibition of adenosine kinase potentiates cardiac adenosine release, *Circ. Res.*, 81, 154, 1997.
4. Ueland, P.M., Pharmacological and biochemical aspects of S-adenosylhomocysteine and S-adenosylhomocysteine hydrolase, *Pharmacol. Rev.*, 34, 223, 1982.
5. Blay, J., White, T.D., and Hoskin, D.W., The extracellular fluid of solid carcinomas contains immunosuppressive concentrations of adenosine, *Cancer Res.*, 57, 2602, 1997.
6. Ethier, M.F. and Dobson, J.G. Jr., Adenosine stimulation of DNA synthesis in human endothelial cells, *Am. J. Physiol.*, 272, 1470, 1997.
7. MacLaughlin, M. et al., Adenosine activates mesangial cell proliferation, *Cell Signal.*, 9, 59, 1997.
8. Fishman, P., Bar-Yehuda, S., and Vagman, L., Adenosine and other low molecular weight factors released by muscle cells inhibit tumor cell growth, *Cancer Res.*, 58, 3181, 1998.
9. Barry, C.P. and Lind, S.E., Adenosine-mediated killing of cultured epithelial cancer cells, *Cancer Res.*, 60, 1887, 2000.
10. Brown, J.R., Cornell. K., and Cook, P.W., Adenosine- and adenine-nucleotide-mediated inhibition of normal and transformed keratinocyte proliferation is dependent upon dipyridamole-sensitive adenosine transport, *J. Invest. Dermatol.*, 115, 849, 2000.
11. Tanaka, Y. et al., Apoptosis induced by adenosine in human leukemia HL-60 cells, *Exp. Cell. Res.*, 213, 242, 1994.
12. Fishman, P. et al., Adenosine acts as an inhibitor of lymphoma cell growth: a major role for the A3 adenosine receptor, *Eur. J. Cancer*, 36, 1452, 2000.
13. Tey, H.B., Khoo, H.E., and Tan, C.H., Adenosine modulates cell growth in human epidermoid carcinoma (A431) cells, *Biochem. Biophys. Res. Commun.*, 187, 1486, 1992.
14. Bajaj, S. et al., Adenosine and adenosine analogues are more toxic to chronic lymphocytic leukemia than to normal lymphocytes, *Blood*, 62, 75, 1983.
15. Sandberg, G. and Fredholm, B.B., Regulation of thymocyte proliferation: effects of L-alanine, adenosine and cyclic AMP in vitro, *Thymus*, 3, 63, 1981.
16. Rozengurt, E., Adenosine receptor activation in quiescent Swiss 3T3 cells: enhancement of cAMP levels, DNA synthesis and cell division, *Exp. Cell. Res.*, 139, 71, 1982.
17. Dusseau, J.W. and Hutchins, P.M., Hypoxia-induced angiogenesis in chick chorioallantoic membranes: a role for adenosine, *Respir. Physiol.*, 71, 33, 1988.
18. Pospisil, M. et al., Elevation of extracellular adenosine induces radioprotective effects in mice, *Radiat. Res.*, 134, 32, 1993.
19. Ohigashi, T., Brookins, J., and Fisher, J.W., Adenosine A1 receptors and erythropoietin production, *Am. J. Physiol.*, 265, 934, 1993.
20. DAncona, S. et al., Effect of dipyridamole, 5-(N-ethyl)-carboxamidoadenosine and 1,3-dipropyl-8-(2-amino-4-chlorophenyl)-xanthine on LOVO cell growth and morphology, *Anticancer Res.*, 14, 93, 1994.
21. Zink, A. et al. Adenosine A1-receptors inhibit cAMP and Ca^{2+} mediated calcitonin secretion in C-cells, *Horm. Metab. Res.*, 27, 408, 1995.
22. Woodhouse, E.C. et al., Adenosine receptor mediates motility in human melanoma cells, *Biochem. Biophys. Res. Commun.*, 246, 888, 1998.
23. Khoo, H.E. et al., Differential expression of adenosine A1 receptors in colorectal cancer and related mucosa, *Cancer Lett.*, 106, 17, 1996.
24. Auchampach, J.A. et al., Canine mast cell adenosine receptors: cloning and expression of the A3 receptor and evidence that degranulation is mediated by the A2B receptor, *Mol. Pharmacol.*, 52, 846, 1997.

3999_C018.fm Page 311 Tuesday, April 11, 2006 8:23 PM

25. Gessi, S. et al., A(3) adenosine receptors in human neutrophils and promyelocytic HL60 cells: a pharmacological and biochemical study, *Br. J. Pharmacol.*, 134, 116, 2001.

26. Merighi, S. et al., Pharmacological and biochemical characterization of adenosine receptors in the human malignant melanoma A375 cell line, *Br. J. Pharmacol.*, 134, 1215, 2001.

27. Suh, B.C. et al., Pharmacological characterization of adenosine receptors in PGT-beta mouse pineal gland tumour cells, *Br. J. Pharmacol.*, 134, 132, 2001.

28. Trincavelli, M.L. et al., A3 adenosine receptors in human astrocytoma cells: agonist-mediated desensitization, internalization, and down-regulation, *Mol. Pharmacol.*, 62, 1373, 2002.

29. Madi, L. et al., The A3 adenosine receptor is highly expressed in tumor versus normal cells: potential target for tumor growth inhibition, *Clin. Cancer Res.*, 10, 4472, 2004.

30. Panjehpour, M. and Karami-Tehrani, F., An adenosine analog (IB-MECA) inhibits anchorage-dependent cell growth of various human breast cancer cell lines, *Int. J. Biochem. Cell Biol.*, 36, 1502, 2004.

31. Gessi, S. et al., Elevated expression of A_3 adenosine receptors in human colorectal cancer is reflected in peripheral blood cells, *Clin. Cancer Res.*, 10, 5895, 2004.

32. Fishman, P. et al., The A_3 adenosine receptor as a new target for cancer therapy and chemoprotection, *Exp. Cell Res.*, 269, 230, 2001.

33. Fishman, P. et al., Evidence for involvement of Wnt signaling pathway in IB-MECA mediated suppression of melanoma cells, *Oncogene*, 21, 4060, 2002.

34. Ohana, G. et al., Inhibition of primary colon carcinoma growth and liver metastasis by the A_3 adenosine receptor agonist CF10, *Br. J. Cancer*, 89, 1552, 2003.

35. Fishman, P. et al., Targeting the A_3 adenosine receptor for cancer therapy: inhibition of prostate carcinoma cell growth by A3AR agonist, *Anticancer Res.*, 23, 2077, 2003.

36. Madi. L. et al., A_3 adenosine receptor activation in melanoma cells: association between receptor fate and tumor growth inhibition, *J. Biol. Chem.*, 278, 42121, 2003.

37. Ohana, G. et al., Differential effect of adenosine on tumor and normal cell growth: focus on the A_3 adenosine receptor, *J. Cell. Physiol.*, 186, 19, 2001.

38. Bar-Yehuda, S. et al., Agonists to the A_3 adenosine receptor induce G-CSF production via NF-kappaB activation: a new class of myeloprotective agents, *Exp. Hematol.*, 30, 1390, 2002.

39. Kohno, Y. et al., Induction of apoptosis in HL-60 human promyelocytic leukemia cells by adenosine A(3) receptor agonists, *Biochem. Biophys. Res. Commun.*, 219, 904, 1996.

40. Yao, Y. et al., Adenosine A_3 receptor agonists protect HL-60 and U-937 cells from apoptosis induced by A_3 antagonists, *Biochem. Biophys. Res. Commun.*, 232, 317, 1997.

41. Kim, S.G. et al., p53-Independent induction of Fas and apoptosis in leukemic cells by an adenosine derivative, Cl-IB-MECA, *Biochem. Pharmacol.*, 63, 871, 2002.

42. Panjehpour, M. and Karami-Tehrani, F., An adenosine analog (IB-MECA) inhibits anchorage-dependent cell growth of various human breast cancer cell lines, *Int. J. Biochem. Cell Biol.*, 36, 1502, 2004.

43. Lu, J., Pierron, A., and Ravid, K., An adenosine analogue, IB-MECA, down-regulates estrogen receptor alpha and suppresses human breast cancer cell proliferation, *Cancer Res.*, 63, 6413, 2003.

44. Merighi, S. et al., A_3 adenosine receptor activation inhibits cell proliferation via phosphatidylinositol 3-kinase (PI3K)/AKT-dependent inhibition of the extracellular signal-regulated kinase (ERK)1/2 phosphorylation in A375 human melanoma cells, *J. Biol. Chem.*, 2005.

PE/AU: Ref. nos. 30 and 42 are identical.

AU: Ref. nos. 42 and 30 are identical. Please delete one and renumber all following references in text and in reference list.Or substitute another reference for one or the other.

45. Fang, X. et al., Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A, *Proc. Natl. Acad. Sci. U.S.A.*, 97, 11960, 2000.
46. Ferkey, D.M., and Kimelman, D., GSK-3: new thoughts on an old enzyme, *Dev. Biol.* 225, 471, 2000.
47. Davies, G., Jiang, W.G., and Mason, M.D., The interaction between beta-catenin, GSK3beta and APC after mItogen induced cell-cell dissociation, and their involvement in signal transduction pathways in prostate cancer, *Int. J. Oncol.*, 18, 843, 2001.
48. Zhong, H., Voll, R.E., and Ghosh, S., Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300, *Mol. Cell*, 1,661, 1998.
49. Lindholm, P.F. et al., The role of constitutive NF-kappa B activity in PC-3 human prostate cancer cell invasive behavior, *Clin. Exp. Metastasis*, 18, 471, 2000.
50. Hoskin, D.W. et al., Adenosine acts through an A_3 receptor to prevent the induction of murine anti-CD3-activated killer T cells, *Int. J. Cancer.*, 99, 386, 2002.
51. MacKenzie, W.M., Hoskin, D.W., and Blay, J., Adenosine inhibits the adhesion of anti-CD3-activated killer lymphocytes to adenocarcinoma cells through an A3 receptor, *Cancer Res.*, 54, 3521, 1994.
52. MacKenzie, W.M., Hoskin, D.W., and Blay, J., Adenosine suppresses alpha(4)beta(7) integrin-mediated adhesion of T lymphocytes to colon adenocarcinoma cells, *Exp. Cell. Res.*, 276, 90, 2002.
53. Smith, S.R., Denhardt, G., and Terminelli, C., A role for histamine in cytokine modulation by the adenosine A(3) receptor agonist, 2-Cl-IB-MECA, *Eur. J. Pharmacol.*, 457,57, 2002.
54. Harish, A. et al., A_3 adenosine receptor agonist potentiates natural killer cell activity, *Int. J. Oncol.*, 23, 1245, 2003.
55. Bar-Yehuda, S. et al., CF101, an agonist to the A_3 adenosine receptor, enhances the chemotherapeutic effect of 5-fluorouracil in a colon carcinoma murine model, *Neoplasia*, 7, 85, 2005.
56. Fishman, P. et al., A_3 adenosine receptor as a target for cancer therapy, *Anti-Cancer Drugs*, 13, 437, 2002.
57. Merighi, S. et al., Pyrazolotriazolopyrimidine derivatives sensitize melanoma cells to the chemotherapic drugs: taxol and vindesine, *Biochem. Pharmacol.*, 66,739, 2003.
58. Fishman, P. et al., Adenosine acts as a chemoprotective agent by stimulating G-CSF production: a role for A_1 and A_3 adenosine receptors, *J. Cell. Physiol.*, 183, 393, 2000.
59. Shneyvays, V. et al., Activation of A(3)adenosine receptor protects against doxorubicin-induced cardiotoxicity, *J. Mol. Cell. Cardiol.*, 33, 1249, 2001.