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Research Overview

Modulation of the A_3 Adenosine Receptor by Low Agonist Concentration Induces Antitumor and Myelostimulatory Effects

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Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT The A_3 adenosine receptor (A_3AR) agonists IB-MECA and Cl-IB-MECA act as anticancer agents by inhibiting the growth of melanoma and colon carcinoma cells both in vitro and in vivo. The A_3AR is highly expressed in these tumor cells and upon agonist binding receptor internalization and recycling occurs. The downstream signaling pathways that follow these events include a decrease in the expression level of protein kinase A (PKA) and protein kinase B/Akt (PKB/Akt) leading to the deregulation of the Wnt and the NF-κB signaling pathways. Interestingly, IB-MECA and Cl-IB-MECA generate the opposite, beneficial effect on myeloid cells. These agonists induce the production of granulocyte colony-stimulating factor (G-CSF) by murine splenocytes. The molecular mechanisms underlying the events prior to G-CSF production include the upregulation of NF-κB and the upstream kinases phosphoinositide 3-kinase (PI3K), PKB/Akt, and IKK. In addition, a myeloprotective effect is noted upon administration of IB-MECA and Cl-IB-MECA to chemotherapy-treated mice. This was expressed by accelerated recovery of white blood cells and neutrophil counts in cyclophosphamide-treated mice following agonist administration. Taken together, activation of the A_3AR on tumor and normal cells generates opposing responses: in tumor cells, NF-κB expression level decreases, resulting in tumor growth inhibition, while in normal cells it is upregulated, leading to G-CSF production and the induction of myelostimulation. It thus appears that A_3AR agonists act as tumor growth inhibitors, simultaneously maintaining the myeloid system, capable of preventing the damage of chemotherapeutic agents. Drug Dev. Res. 58:386–389, 2003.

Key words: A_3 adenosine receptor; cancer; myelostimulation; chemotherapy

INTRODUCTION

Chemotherapy, the mainstay of cancer therapy, is applied in various conditions, including eradication of micrometastases in the neo-adjuvant setup, down-sizing and down-staging of primary masses before surgery or radiation therapy, and for palliation of symptomatic metastatic disease. This treatment is based on the administration of cytotoxic agents such as doxorubicin, taxanes, platinum compounds, or antimetabolites and is aimed to suppress tumor cell development. The main limitation of chemotherapy is the associated dose-limiting toxicity and a wide spectrum of side effects, mild and temporary or chronic / life threatening. Nausea, vomiting, hair loss,

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and myelosuppression are the most common toxic effects of chemotherapy. Granulocyte colony-stimulating factor (G-CSF), a hematopoietic growth factor, has become a standard supportive therapy for cancer patients with myelosuppression or, more specifically, neutropenia. It stimulates the proliferation and differentiation of myeloid progenitors, leading to a reduction in the incidence of febrile neutropenia and acceleration of neutrophil recovery after chemotherapy or bone marrow transplantation [Brandt et al., 1988]. Specific targeting of tumor cells by chemotherapy while sparing normal and healthy body tissues is under continuous investigation.

Recently, it has been observed that striated muscle tissue rarely harbor metastases. In an attempt to understand this clinical observation, we showed that muscle cells secrete A_3AR agonists that specifically inhibit the growth of various tumor cell types [Djaldetti et al., 1996; Fishman et al., 1998, 2000a,b; Bar Yehuda et al., 1999]. Synthetic agonists to A_3AR such as 1-Deoxy-1-[6-[[(3-iodophenyl)methyl]amino]-9H -purine-9-yl]-N-methyl-β-D-ribofura-nuronamide (IB-MECA) and 2-chloro-N^6-(3-iodobenzyl)-adenosine-5′-N-methyl-uronamide (Cl-IB-MECA) were found to exert similar effects [Bar Yehuda et al., 2001]. Interestingly, agonists to the A_3AR-induced the production of G-CSF by murine splenocyte mononuclear cells. The observation that A_3AR agonists inducing both antitumor and myelostimulatory effects led us to further explore the mechanism involved. In addition, the efficacy of the agonists as anticancer and myeloprotective agents in vivo was examined [Fishman et al., 2001; Ohana et al., 2001].

ANTITUMOR EFFECTS OF A_3AR AGONISTS

In Vitro Studies

IB-MECA and Cl-IB-MECA (at nanomolar concentrations) suppressed the proliferation of B16-F10 melanoma and HCT-116 colon carcinoma cells in vitro. This effect was tested utilizing the incorporation of ^{3}[H]-thymidine to proliferating cells and 25–35% cell proliferation inhibition was noted. Cell cycle arrest at the G0/G1 phase was observed, indicating cytostatic activity as part of the antitumor mechanism. The A_3AR antagonist MRS1523 counteracted the effect of IB-MECA and Cl-IB-MECA, suggesting that the inhibition of tumor growth by the agonists was receptor mediated [Fishman et al., 2001].

We then studied the receptor internalization/recycling process that turns the on/off receptor-mediated signal transduction pathway. Confocal microscopy studies revealed that B16-F10 melanoma cells highly exhibited A_3ARs on the cell surface, which was rapidly internalized to the cytosol following activation. Receptor degradation then occurred followed by receptor mRNA and protein resynthesis and its recycling to the cell surface. These events generated the modulation of key signaling proteins including protein kinase A (PKA), protein kinase B (PKB/Akt), and the key component of the Wnt signaling pathway, glycogen synthase kinase-3β (GSK-3β), known to phosphorylate the cytosolic protein, β-catenin. The latter is ubiquitinated upon phosphorylation, shifting cells toward cell cycle arrest or apoptosis. Failure of GSK-3β to phosphorylate β-catenin leads to its accumulation in the cytoplasm and its translocation to the nucleus. In association with Lef/TCF, transcription of the cell cycle genes c-Myc and cyclin D1 takes place [Ferkey et al., 2000; Morin, 1999; Novak and Dedhar, 1999]. The activity of GSK-3β is controlled by the two kinases PKA and PKB/Akt which, upon phosphorylation, induce inactivation of the enzyme. Our studies showed that activation of the A_3AR in B16-F10 melanoma cells led to downregulation of PKA and PKB/Akt. As a result, GSK-3β was not phosphorylated and retained its active form, enabling the phosphorylation and downregulation of β-catenin. Consequently, cyclin D1 and c-Myc decreased and inhibition of cell cycle progression was shown [Fishman et al., 2002]. Interestingly, the antiapoptotic NF-κB, a transcription factor known to be highly expressed in tumor cells, was inhibited upon A_3AR activation. PKB/Akt controls the NF-κB level, indicating that its inhibition leads to NF-κB downregulation (Fig. 1). These data demonstrate that the intercommunication of the A_3AR, Wnt, and NF-κB pathways is apparently driven via PKA and PKB/Akt.

In Vivo Studies

IB-MECA and Cl-IB-MECA were shown to markedly inhibit the growth of B16-F10 melanoma. In tumor lesions derived from IB-MECA-treated mice, A_3AR expression and the level of the key signaling proteins, PKA, GSK-3β, β-catenin, NF-κB, cyclin D1, and c-Myc, were modulated as observed in vitro. This led to the definition of the aforementioned six proteins as "marker proteins," which reflect A_3AR functionality and may serve as indicators of tumor cell response to IB-MECA.

MYELOSTIMULATORY EFFECT OF A_3AR AGONISTS: MECHANISM OF ACTION

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

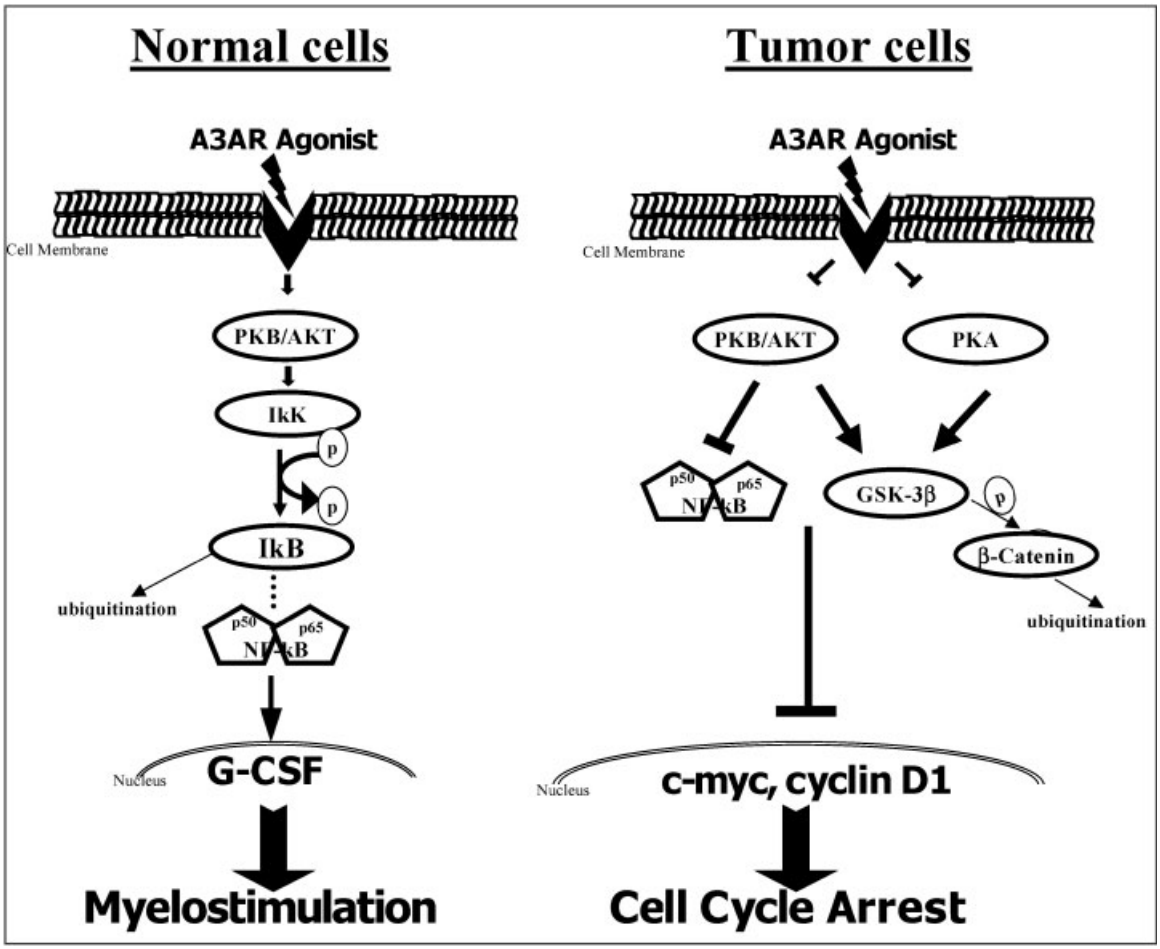

Fig. 1. Schematic presentation of the signaling pathways taking place upon A_3AR activation in normal and tumor cells.

bone marrow colony-forming cells. Splenocytes derived from these mice produced higher G-CSF level than controls. The molecular mechanisms underlying the events prior to G-CSF production included the upregulation of NF-κB and the upstream kinases phosphoinositide 3-kinase (PI3K), PKB/Akt, and IKK (Fig. 1). Accelerated recovery of white blood cells and neutrophil counts were observed in cyclophosphamide treated mice following CF101 administration [Bar-Yehuda et al., 2002].

Thus, the NF-κB signaling pathway also plays a key role in mediating the myeloprotective effect of IB-MECA. In distinction from tumor cells, normal G-CSF-producing cells respond to IB-MECA in an opposite way by elevating NF-κB levels.

CONCLUDING REMARKS

We may thus conclude that at low nanomolar concentrations the agonists bind specifically and exclusively to the A_3AR and activate downstream signaling mechanisms leading to tumor growth inhibition on the one hand and a myelostimulatory effect on the other hand. At higher concentrations, nonspecific mechanisms may be generated either via other adenosine receptors or through direct initiation of other responses (ion channels, Fas or Bax activation, etc.).

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