Adenosine A2B Receptor: From Cell Biology to Human Diseases

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Extracellular adenosine is a ubiquitous signaling molecule that modulates a wide array of biological processes. Recently, significant advances have been made in our understanding of A2B adenosine receptor (A2B AR). In this review, we first summarize some of the general characteristics of A2B AR, and then we describe the multiple binding partners of the receptor, such as newly identified α-actinin-1 and p105, and discuss how these associated proteins could modulate A2B AR's functions, including certain seemingly paradoxical functions of the receptor. Growing evidence indicates a critical role of A2B AR in cancer, renal disease, and diabetes, in addition to its importance in the regulation of vascular diseases, and lung disease. Here, we also discuss the role of A2B AR in cancer, renal disease, and diabetes and the potential of the receptor as a target for treating these three diseases.

Keywords: A2B adenosine receptor, binding proteins, cancer, renal disease, diabetes

INTRODUCTION

Extracellular adenosine is a ubiquitous signaling molecule that modulates a wide array of biological processes. Most of the extracellular adenosine is derived from the release and metabolism of adenine nucleotides such as ATP following diverse stimuli, including mechanical stress, osmotic challenge, inflammation, and tissue injury (Dunwiddie et al., 1997; Fredholm et al., 2001a; Picher et al., 2003, 2004; Eckle et al., 2007; Grenz et al., 2007; Ohta and Sitkovsky, 2014; Ross et al., 2014; Fuentes and Palomo, 2015; Kowal et al., 2015; Borea et al., 2016; Covarrubias et al., 2016; Hamidzadeh and Mosser, 2016). Conversely, extracellular adenosine is eliminated mainly through two mechanisms: one, transport of adenosine back into the cell by nucleoside transporters; and two, deamination of adenosine to inosine by adenosine deaminase (ADA; Blackburn and Kellems, 1996) or phosphorylation of adenosine to AMP by adenosine kinase (Lloyd and Fredholm, 1995; Spychala et al., 1996). The combined actions of these adenosine generation and elimination mechanisms regulate extracellular adenosine levels, which range from 10 to 200 nM under homeostatic conditions but can be elevated to 10–100 µM in hypoxic or stressed environments (Fredholm, 2007).

The biological functions of extracellular adenosine are mediated by four subtypes of adenosine receptors (ARs), A1, A2A, A2B, and A3, each of which presents a unique pharmacological profile, tissue distribution, and effector coupling (Fredholm et al., 2001b). Among human ARs, A1AR, and A3 AR share 49% sequence similarity and A2A AR and A2B AR share 59% similarity (Jacobson and Gao, 2006; Goblyos and Ijzerman, 2009).

Perhaps because A2B AR binds to adenosine with low affinity (EC50 = 24 µM; Beukers et al., 2000; Fredholm et al., 2001b, 2011a), A2B AR is frequently considered to represent a low-affinity
version of A2A AR and to be of comparatively lesser physiological relevance. However, recent advances in pharmacological and molecular tools have allowed researchers to determine that A2B AR can be coupled to distinct intracellular signaling pathways and play physiological roles that differ from those of A2A AR (Yang et al., 2006, 2010a; Grenz et al., 2012; Johnston-Cox et al., 2012; Koupenanova et al., 2012; Eckle et al., 2013; Morello and Miele, 2014; Patel et al., 2014; Tak et al., 2014; Eisenstein et al., 2015; Tang et al., 2015; Vecchio et al., 2016). In this review, we discuss our current understanding of the cellular functions of A2B AR and their implications for the pathogenesis of several human diseases.

Molecular Function and Cellular Localization of A2B AR

A2B AR was first identified and cloned in 1992 by Rivkees and Reppert and by Pierce et al. from the rat hypothalamus (Rivkees and Reppert, 1992) and human hippocampus (Pierce et al., 1992). The proposed structure of A2B AR is the typical G-protein-coupled receptor (GPCR) structure, and the predicted molecular mass of A2B AR is 36–37 kDa (Feoktistov and Biaggioni, 1997).

The major signaling pathway of A2B AR is suggested to be the pathway involving adenyl cyclase (AC) that leads to an increase in intracellular cAMP levels and results in the subsequent activation of PKA and other cAMP effectors such as Epac (Peakman and Hill, 1994; Murakami et al., 2000; Sitaraman et al., 2001; Lyng et al., 2003; Fang and Ohl, 2007; Darashchonak et al., 2014; He et al., 2014). However, the A2B AR-Gq-PLC pathway also mediates several crucial functions of A2B AR (Gao et al., 1999; Linden et al., 1999; Panjehpour et al., 2005), and A2B AR further couples to the MAPK and arachidonic acid signaling pathways and regulates membrane ion channels probably through G-protein βγ subunits (Feoktistov et al., 1999; Jimenez et al., 1999; Schulte and Fredholm, 2003a,b; Donoso et al., 2005).

The recent development of A2B AR-knockout/lacZ-knockin mice has enabled the determination of A2B AR distribution in vivo (Yang et al., 2006); A2B AR is widely expressed in numerous tissues and organs, including the vasculature, aortic vascular smooth muscle, cecum, large intestine, brain, and urinary bladder (Yaar et al., 2005; Wang and Huxley, 2006; Yang et al., 2006). Furthermore, a high level of A2B AR expression has been detected in diverse types of cells, including various immune cells such as mast cells (Hua et al., 2007; Ryzhov et al., 2008b), neutrophils (Eckle et al., 2008a), dendritic cells (Pacheco et al., 2005; Ben Addi et al., 2008; Novitskiy et al., 2008), macrophages (Yang et al., 2006), and lymphocytes (Mirabet et al., 1999; Eckle et al., 2008a), as well as other cell types such as type II alveolar epithelial cells (Cagnina et al., 2009), endothelial cells (Yang et al., 2006), chromaffin cells (Casado et al., 1992), astrocytes (Peakman and Hill, 1994; Jimenez et al., 1999), neurons (Corset et al., 2000; Christofi et al., 2001; Stein et al., 2001), and taste cells (Nishida et al., 2014). Moreover, A2B AR expression is influenced by diverse environmental cues such as inflammation, cell stress, injury, and hypoxia (Xaus et al., 1999; Fredholm et al., 2001a; Kolachala et al., 2005; Kong et al., 2006; Hart et al., 2009; Hasko et al., 2009). For example, previous studies have shown that interferon-γ, a proinflammatory cytokine, increases the A2B AR transcriptional level in mouse macrophage cells (Xaus et al., 1999); TNF-α upregulates A2B AR mRNA and protein levels in human colonic epithelial cells (Kolachala et al., 2005); and other mediators such as LPS (Nemeth et al., 2003), IL-1β (Nguyen et al., 2003), free radicals (St Hilaire et al., 2008), and endogenous adenosine (Sitaraman et al., 2002) also enhance A2B AR expression.

A2B AR Binding Partners and Their Cellular Functions

Identifying the binding partners of A2B AR is crucial for understanding the receptor’s function and regulation. As in other GPCRs, the intracellular portions of A2B AR serve as signal integrators by providing binding sites for effectors or regulatory proteins, although other parts of A2B AR might also be involved in protein interaction. Besides trimeric G proteins and β-arrestin (Feoktistov and Biaggioni, 1997; Mundell et al., 2000; Klinger et al., 2002), the two universal binding partners of GPCRs, numerous other proteins interact with A2B AR. Here, we list these A2B AR binding partners in the order of interaction discovery, and discuss how these proteins modulate or mediate A2B AR functions (Figure 1).

ADA

ADA is an enzyme that catalyzes the hydrolytic deamination of adenosine to inosine. Apart from being present in the cytosol and the nucleus, ADA is anchored to the cell surface by other membrane proteins, including CD26 (Pacheco et al., 2005) and A1 AR (Saura et al., 1998) in various cell/tissue types such as cultured cortical neurons (Ruiz et al., 2000), DDT1MF-2 cells (Ciruela et al., 1996), and pig brain cortical membrane (Saura et al., 1996). In addition to A1 AR and CD26, A2B AR was reported to mediate ADA docking—in CHO and Jurkat cells—onto the extracellular surface (Herrera et al., 2001); counterintuitively, the binding of ADA, even when ADA lacked enzymatic activity, increased the binding affinity of NECA (a nonselective A2 AR agonist) for A2B AR and the subsequent production of cAMP. The interaction between ADA and A2B AR was also confirmed in dendritic cells (Pacheco et al., 2005) and gastric mucosa parietal cells (Arin et al., 2015). In dendritic cells, the ADA-A2B AR complex triggers a cell adhesion-costimulatory signal that promotes an immune response, and this is also independent of ADA enzymatic activity (Pacheco et al., 2005). Thus, the ADA-A2B AR complex appears to perform multiple functions, including modulating agonist binding, promoting cell adhesion/costimulation, and degrading extracellular adenosine.

Deleted in Colorectal Carcinoma (DCC) and Netrin-1

DCC has been proposed to function as a netrin-1 receptor and thus mediate netrin-1-induced axon outgrowth. Corset and
FIGURE 1 | $A_{2B}$AR binding partners and their cellular functions.

collaborators identified $A_{2B}$AR as one of the proteins that directly binds to DCC and functions as a netrin-1 coreceptor, because netrin-1 activated $A_{2B}$AR and induced cAMP production, and further suggested that $A_{2B}$AR is the central mediator of netrin signaling in the regulation of the outgrowth of dorsal spinal cord axons (Corset et al., 2000). However, a subsequent study argued against this view (Stein et al., 2001): the DCC ectodomain was found to interact directly with netrin-1 and mediate netrin signaling to regulate axon growth, and the results of pharmacological analyses suggested that $A_{2B}$AR function was not required for netrin-1-induced axon growth and guidance. Thus, DCC was proposed to mediate netrin signaling in axon growth and guidance independently of $A_{2B}$AR activation (Stein et al., 2001). Intriguingly, more recent studies have reported that netrin-1 attenuates neutrophil transmigration and hypoxia-induced inflammation (Rosenberger et al., 2009), alveolar fluid clearance (He et al., 2014), and diabetic nephropathy (Tak et al., 2013) and induces cancer-cell invasion (Rodrigues et al., 2007) in an $A_{2B}$AR-dependent manner. These results appear to support the general notion that $A_{2B}$AR mediates the function of netrin-1 at least in certain tissues. Further investigation is required to clarify the discrepancy between the aforementioned studies.

**E3KARP-EZRIN-PKA AND SNARE**

Sitaraman and colleagues demonstrated that the majority of $A_{2B}$AR localizes intracellularly in quiescent cells and is recruited to the plasma membrane upon agonist stimulation (Sitaraman et al., 2002). The SNARE protein SNAP-23 directly interacts with human $A_{2B}$AR and participates in $A_{2B}$AR recruitment to the plasma membrane (Wang et al., 2004), and following SNARE-dependent translocation to the plasma membrane, human $A_{2B}$AR directly associates with E3KARP (NHERF2) and ezrin and forms a multiprotein complex (Sitaraman et al., 2002). Ezrin is a PKA-anchoring protein, or AKAP, that associates with the actin cytoskeleton (Sun et al., 2000), and this multiprotein complex not only anchors $A_{2B}$AR to the plasma membrane, but also stabilizes $A_{2B}$AR expression in the plasma membrane. Furthermore, compartmentalized PKA is effectively activated by $A_{2B}$AR-induced cAMP production, and the PKA thus activated stimulates CFTR-mediated chloride secretion; this model is consistent with the functional evidence obtained in an early study (Huang et al., 2001).

Interestingly, at its C-terminal end, human $A_{2B}$AR contains a type 2 PDZ-binding motif (XΦXΦ), GVGL, but not a type 1 PDZ-binding motif (X5/TXV/L). Sitaraman et al. speculated that a PDZ-binding-motif-like sequence in the 3rd intracellular loop in $A_{2B}$AR might mediate the interaction with E3KARP, a PDZ-domain-containing protein (Sitaraman et al., 2002). However, recent studies indicate that the GVGL sequence of $A_{2B}$AR participates in the trafficking and surface expression of $A_{2B}$AR (Watson et al., 2011, 2016), possibly by binding to a PDZ-domain-containing protein. Further investigation is required to determine whether
GVGL binds to E3KARP or another PDZ-domain-containing protein.

**A2AR**

The function and trafficking of several GPCRs are affected by the heterooligomerization of these receptors. Moriyama and Sitkovsky reported that A2AR coexpression with A2BAR improves the cell-surface expression of A2BAR, which is normally poor because A2BAR lacks a dominant forward-transport signal for export from the ER to the cell surface (Moriyama and Sitkovsky, 2010). The study further suggested that the functional interaction between A2AR and A2BAR might be a consequence of their physical association (Moriyama and Sitkovsky, 2010), but how these two receptors interact was not explored. Because both A2AR and A2BAR were shown to interact with actinins in one previous study (in which the specific actinin isoform was not identified; Burgueno et al., 2003) or with α-actinin-1 in another study (Sun et al., 2016), the α-actinin-1 homodimer or a heterodimer of α-actinin-1 with another actinin isoform might mediate the dimerization of A2AR and A2BAR and thus promote the surface expression of A2AR. This mechanism is clearly not mutually exclusive with the mechanism by which α-actinin-1 mediates A2BAR interaction with actin filaments and thereby modulates the trafficking and surface expression of A2BAR (Sun et al., 2016).

**TRANSCRIPTION FACTOR NFκB1/P105**

NFκB1/p105 is a member of the NFκB family of proteins that perform regulatory functions in diverse biological processes such as inflammation and cell survival and differentiation, as well as in various diseases, including cancer (Barkett and Gilmore, 1999; Hatada et al., 2000; Perkins and Gilmore, 2006). Sun et al. reported that the C-terminal tail of A2BAR binds to NFκB1/p105 independently of ligand activation (Sun et al., 2012). Intriguingly, A2BAR binding to specific sites on p105 prevents the polyubiquitination and degradation of p105 protein and thereby inhibits NFκB activation and reduces inflammation (Sun et al., 2012). In previous studies, both pro- and anti-inflammatory activities have been associated with A2BAR (Blackburn et al., 2009), and the work by Sun et al. potentially sheds light on this paradox: although A2BAR activation by adenosine produces proinflammatory effects, A2BAR can also induce adenosine-independent downregulation of the proinflammatory response by associating with p105. Such receptor bifunctionality displayed by A2BAR—mediation of diametrically opposite effects in the presence and absence of ligand—is reminiscent of dependence receptors (Thibert and Fombonne, 2010). GPCRs other than A2AR have previously been shown to signal through G-protein-independent pathways, including pathways involving transcription factors (Nehring et al., 2000; White et al., 2000). The study of Sun et al. further suggests that the C-terminus of A2BAR potentially provides a target for developing peptidomimetic drugs that block NFκB signaling, which could be used for treating NFκB-related diseases such as inflammation and cancer (Sun et al., 2012).

**α-ACTININ-1**

Actinins, or α-actinins, represent a family of ubiquitously expressed actin-filament-crosslinking proteins. In addition to performing their critical function of actin-filament crosslinking, actinins link membrane receptors, and cell adhesion proteins to actin filaments and thereby modulate the function and trafficking of these membrane proteins (Oikonomou et al., 2011; Foley and Young, 2014). A recent study by Sun and colleagues suggested that α-actinin-1 binds to the A2BAR C-terminus and stabilizes the receptor’s global and cell-surface expression (Sun et al., 2016), which revealed a previously unidentified molecular mechanism for controlling the cellular levels of A2BAR. Because the actinin-1 isoform investigated in the study was the Ca²⁺-sensitive exon19a splice variant, an intriguing question is whether actinin-1-dependent regulation of A2BAR is also Ca²⁺ sensitive under physiological conditions.

In contrast to α-actinin-1, α-actinin-4, another highly homologous non-muscle actinin isoform, did not interact with A2BAR (Sun et al., 2016). Interestingly, α-actinin-4 has been suggested to interact with the NFκB subunits p65 and p50 and function as a coactivator of the transcription factor NFκB (Zhao et al., 2015). Thus, future studies could investigate whether actinin-1 also associates with NFκB proteins, including p105, and how this association affects the interaction between p105 and A2BAR.

**A2BAR IN HUMAN DISEASES**

Numerous studies have demonstrated a critical role of A2BAR in the regulation of vascular diseases (Martin, 1992; Dubey et al., 1996; Yang et al., 2008, 2010a), chronic lung disease (Sun et al., 2006; Wilson et al., 2009; Zhou et al., 2009; Zaynagetdinov et al., 2010), and acute lung injury (Eckle et al., 2008a,b; Schingnitz et al., 2010), and several excellent reviews have summarized these studies (Spicuzza et al., 2006; Hasko et al., 2009; Aherne et al., 2011; Headrick et al., 2013). Therefore, in this review, we discuss only the potential functions of A2BAR in three other common human diseases, cancer, renal disease, and diabetes (Figure 2).

**A2BAR IN CANCER**

Growing evidence indicates that A2BAR potentially plays a pathophysiological role in human cancer and might serve as a target for novel therapies or cotherapies for cancer. The possible functions of A2BAR in tumor progression and metastasis are discussed here.

First, A2BAR is highly expressed in various types of tumor cells or tissues and promotes tumor-cell proliferation. For instance, A2BAR was found to be overexpressed in colorectal carcinoma cells and tissues, and inhibition of A2BAR blocked the proliferation of colon cancer cells (Ma et al., 2010). In prostate cancer, A2BAR increased cancer-cell proliferation in both ligand-dependent, and ligand-independent manners (Wei et al., 2013; Vecchio et al., 2016). In human oral cancer, A2BAR was shown to be upregulated in oral squamous carcinoma cells, and A2BAR knockdown reduced the proliferation of oral cancer cells through HIF-1α activation (Kasama et al., 2015). Moreover, A2BAR was
reported to foster bladder and breast tumor growth in syngeneic mice (Cekic et al., 2012).

Second, A2B AR modulates tumor-cell metastasis. A2B AR was implicated in promoting breast cancer cell migration in vitro and lung metastasis in vivo (Stagg et al., 2010; Desmet et al., 2013), although the underlying molecular mechanism was not fully elucidated. However, the results of a subsequent study suggested a possible explanation: A2B AR activation suppressed the prenylation of the small GTPase Rap1B and diminished Rap1B-mediated cell adhesion, which promoted cell migration (Ntantie et al., 2013).

Third, A2B AR might regulate the tumor microenvironment, including the surrounding blood vessels, immune cells, fibroblasts, and the extracellular matrix. Ryzhov and colleagues provided the first genetic evidence indicating that A2B AR regulates vascular endothelial growth factor (VEGF) production from tumor-infiltrating host immune cells and thereby promotes tumor growth (Ryzhov et al., 2008a). Concomitantly, other groups suggested that A2B AR alters angiogenesis by regulating the production of a wide array of pro- or anti-angiogenic factors such as basic fibroblast growth factor (bFGF), angiopoietin2, and a subset of cytokines (Feoktistov et al., 2002, 2003; Merighi et al., 2009). In addition to affecting angiogenesis, A2B AR regulates dendritic-cell differentiation and function (Novitskiy et al., 2008; Yang et al., 2010b) and alternative macrophage activation (Csoka et al., 2012) and thus contributes to cancer progression.

Thus, A2B AR exerts various effects on tumor progression and metastasis. Notably, most of the aforementioned evidence was collected using in vitro systems, and it is critical to further confirm the role of A2B AR in cancer by using in vivo models before A2B AR is used as a potential cancer therapeutic target.

**A2B AR IN RENAL DISEASE**

Renal diseases are estimated to affect millions of people worldwide, whose numbers are growing at a rate of approximately 5–8% annually (Hamer and El Nahas, 2006). Several studies have indicated a critical role of A2B AR in mediating the progression of diabetic nephropathy. Patel et al. and Valladares et al. observed that inhibition of A2B AR activation suppressed VEGF production in glomeruli and further attenuated renal dysfunction in diabetic nephropathy; these data suggested a protective role of A2B AR antagonists in VEGF-induced diabetic nephropathy (Valladares et al., 2008;
Patel and Thaker, 2014). However, this view was challenged by Tak et al., who reported elevated VEGF levels in diabetic A2B-AR-knockout mice (Tak et al., 2014); concordantly, diabetic nephropathy was highly severe in mice with global or vascular endothelial tissue-specific A2B-AR deletion, but not in mice with tubular-epithelial A2B-AR deletion. Therefore, Tak et al. suggested that vascular A2B-AR signaling is the key mediator of kidney protection during diabetic nephropathy (Tak et al., 2014). The methods used and the specific tissues studied by the aforementioned groups were distinct, which might explain their conflicting observations on the role of A2B-AR during diabetic nephropathy. Moreover, the different time windows in which A2B-AR inhibition was induced pharmacologically and genetically might also contribute to the discrepancy in the results (Eisenstein et al., 2015).

In addition to playing a role in diabetic nephropathy, A2B-AR has been suggested, based on studies on several mouse models, to protect against renal fibrosis. In ADA-deficient mice, a high level of adenosine in kidney tissues resulted in proteinuria and renal fibrosis, and treatment with A2B-AR antagonists attenuated renal dysfunction and fibrosis (Dai et al., 2011). Moreover, genetic deletion of A2B-AR protected against renal fibrosis in both mice infused with angiotensin II and mice subjected to unilateral ureteral obstruction (Dai et al., 2011). Furthermore, renal biopsy samples from patients with chronic kidney disease (CKD) showed higher levels of A2B-AR expression than did samples from patients without CKD (Zhang et al., 2013). All of these data suggest that A2B-AR could serve as a potential therapeutic target in the treatment of CKD.

Acute kidney injury, a devastating kidney disease, is often caused by renal ischemia. Rigorous studies from different laboratories have suggested a pivotal role of A2B-AR in acute kidney injury. For example, Grenz et al. used genetic and pharmacological approaches to reveal a role of A2B-AR in protecting against renal injury resulting from ischemia, although the underlying molecular mechanism was not fully clarified (Grenz et al., 2008). Subsequently, the same group proposed two possible explanations for how A2B-AR might provide renal protection: one, A2B-AR reduces neutrophil-dependent TNF-α production and suppresses inflammation (Grenz et al., 2012b); and two, A2B-AR promotes optimal postischemic blood flow within the kidney and thereby ensures the maximal return of blood flow, tissue oxygenation, and removal of waste products from the ischemic kidney through the A2B-AR-ENT1 (equilibrative nucleoside transporter) pathway (Grenz et al., 2012a).

**A2B-AR in Diabetes**

Diabetes mellitus (DM) is the most common endocrine disorder; in 2014, 9% of all adults aged 18+ years were estimated to have diabetes (WHO, 2014), and by 2025, 300 million people worldwide will have the disease (Mane et al., 2012). Adenosine has long been recognized to affect insulin secretion and glucose homeostasis by activating the four AR subtypes (Dong et al., 2001; Nemeth et al., 2007; Fredholm et al., 2011b; Koupenanova and Ravid, 2013; Andersson, 2014; Antonioli et al., 2015). Recently, A2B-AR in particular has been suggested to function as a critical regulator in DM (Rusing et al., 2006; Johnston-Cox et al., 2012, 2014; Eisenstein et al., 2015; Merighi et al., 2015; Wen et al., 2015).

In a type I DM model, the nonselective receptor agonist NECA blocked diabetes development, and this appeared to be mediated by A2B-AR-dependent suppression of proinflammatory cytokine production (Nemeth et al., 2007). These data suggest that A2B-AR represents a potential target for the treatment of type I diabetes.

Conversely, some of the evidence obtained using a type II DM model indicated that A2B-AR plays a pro-diabetic role. Figler et al. suggested that A2B-AR activation increases insulin resistance by elevating the production of proinflammatory mediators such as IL-6 and C-reactive protein (Figler et al., 2011). Deletion of the A2B-AR gene and selective blockade of A2B-AR in mice reduced hepatic glucose production and enhanced glucose disposal into skeletal muscle and brown adipose tissue (Figler et al., 2011). By contrast, other studies suggested an anti-diabetic role of A2B-AR. Johnston-Cox and colleagues showed that A2B-AR plays an essential role in high fat diet (HFD)-induced insulin resistance in mice, and mice lacking A2B-AR displayed diminished glucose clearance and elevated insulin resistance and inflammatory cytokine production (Johnston-Cox et al., 2012). The underlying cellular mechanism here is mediated by A2B-AR expressed in macrophages: reinstatement of macrophage A2B-AR in A2B-AR-null mice restored HFD-induced insulin tolerance and tissue insulin signaling to the level in control mice. The molecular mechanism involves A2B-AR altering cAMP signaling and the levels of macrophage cytokine expression and secretion, and this regulates the levels of insulin receptor-2 and downstream insulin signaling (Johnston-Cox et al., 2014). Similar results were obtained by Csoka et al. (2014), who suggested that A2B-AR plays a crucial role in sustaining glucose homeostasis and preventing insulin resistance under normal dietary conditions by regulating alternative macrophage activation. Insulin- and glucose-induced glucose clearance was impaired in A2B-AR-knockout mice that were fed chow diet, and these knockout mice also exhibited a low level of physical activity, which might contribute to decreased insulin sensitivity in skeletal muscles. Csoka et al. also highlighted the complex role of A2B-AR in regulating liver metabolism (Csoka et al., 2014).

**CONCLUSION**

In this review, we have discussed certain general characteristics of A2B-AR and have described multiple binding partners of the receptor, including α-actinin-1 and p105, whose interactions with the receptor were discovered recently. This identification of A2B-AR-binding proteins will undoubtedly help enhance our understanding of the molecular and cellular functions of A2B-AR; however, to date, fewer binding partners have been reported for A2B-AR than for other AR subtypes. Several reasons might account for this: (1) Little attention was previously devoted to A2B-AR because the receptor was long assumed, inaccurately, to be of lesser physiological relevance as compared with other ARs;
studies on A2B AR were hampered by a lack of useful biological tools such as specific agonists; and (3) novel experimental approaches such as mass spectrometry were not used to identify A2B AR binding partners.

Recent studies have considerably advanced our understanding of the critical role of A2B AR in the pathogenesis of human diseases, and this raises the possibility that A2B AR could be used as a potential target in the treatment of cancer, diabetes, or other diseases. However, opposing functions of A2B AR have been identified in several diseases. For example, A2B AR activation produces pro- and anti-tumoral effects and the receptor performs pro- and anti-inflammatory functions. These paradoxical effects are least partly contributed by the incompletely explored, agonist-independent activities of A2B AR, including its interactions with p105 (Sun et al., 2012), netrin-1 (Corset et al., 2000), ADA (Herrera et al., 2001; Pacheco et al., 2005), or other effector proteins in specific contexts. Moreover, the discrepant effects might be ascribed to different systems and conditions used for studying them, including cell types, animal models, time window of modulation of A2B AR activity, and the potential side effects of given agonists or antagonists. From a clinical perspective, these opposite effects of A2B AR make it highly challenging to decide whether agonists or antagonists should be used in pharmacological interventions for a given disease. Therefore, to effectively use A2B AR as a therapeutic target, studies must be conducted to elucidate precisely how A2B AR agonist-dependent and -independent functions modulate a particular pathological condition in a specific cellular setting and time window.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

Aherne, C. M., Kewley, E. M., and Eltzschig, H. K. (2011). The resurgence of A3 adenosine receptor signaling. Biochim. Biophys. Acta 1808, 1329–1339. doi: 10.1016/j.bbamem.2010.05.016

Anderson, O. (2014). Role of adenosine signalling and metabolism in beta-cell regeneration. Exp. Cell Res. 321, 3–10. doi: 10.1016/j.yexcr.2013.11.019

Antonioli, L., Blandizzi, C., Csoka, B., Pacher, P., and Hasko, G. (2015). Adenosine signalling in diabetes mellitus—pathophysiology and therapeutic considerations. Nat. Rev. Endocrinol. 11, 228–241. doi: 10.1038/nrendo.2015.10

Arin, R. M., Vallejo, A. I., Rueda, Y., Fresnedo, O., and Ochoa, B. (2015). Modulation of murine dendritic cell function by adenine nucleotides and -deaminase in mice. Prog. Nucleic Acid Res. Mol. Biol. 95, 195–226. doi: 10.1016/S0079-6603(08)60194-4

Barkett, M., and Gilmore, T. D. (1999). Control of apoptosis by Rel/NF-κB actin-binding protein alpha-actinin. J. Biol. Chem. 278, 37545–37552. doi: 10.1074/jbc.M302809200

Ben Addi, A., Lefort, A., Hua, X., Libert, F., Communi, D., Ledent, C., et al. (2008). Adenosine A2B receptor blockade slows growth of bladder and breast tumors. J. Immunol. 188, 198–205. doi: 10.4049/jimmunol.1101845

Bernt, J. R., Zhang, H., Yu, J. G., Guzman, J., Xue, J., Kim, M., et al. (2001). Differential gene expression of adenosine A1, A2A, A3, and A2B receptors in the human enteric nervous system. J. Comp. Neurol. 439, 46–64. doi: 10.1002/cne.1334

Canela, E. I., et al. (2003). The adenosine A2A receptor interacts with the actin-binding alpha-actinin. J. Biol. Chem. 278, 37545–37552. doi: 10.1074/jbc.M302809200

Cagnina, R. E., Ramos, S. I., Marshall, M. A., Wang, G., Frazier, C. R., and Linden, J. (2009). Adenosine A2B receptors are highly expressed on murine type II alveolar epithelial cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 297, L647–L744. doi: 10.1152/ajplung.90553.2008

Casado, V., Casillas, T., Mallol, J., Canela, E. I., Lluis, C., and Franco, R. (1992). The adenosine receptors present on the plasma membrane of chromaffin cells are of the A2B subtype. J. Neurochem. 59, 425–431. doi: 10.1111/j.1471-4159.1992.tb09388.x

Celic, C., Sag, D., Li, Y., Theodorescu, D., Strieter, R. M., and Linden, J. (2012). Adenosine A2B receptor blockade slows growth of bladder and breast tumors. J. Immunol. 188, 198–205. doi: 10.4049/jimmunol.1101845

Christofi, F. L., Zhang, H., Yu, J. G., Guzman, J., Xue, J., Kim, M., et al. (2001). Adenosine receptor interactions and -independent functions modulate a particular pathological condition in a specific cellular setting and time window.

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Feoktistov, I., Ryzhov, S., Goldstein, A. E., and Biaggioni, I. (2003). Mast
Feoktistov, I., and Biaggioni, I. (1997). Adenosine A
Feoktistov, I., Goldstein, A. E., and Biaggioni, I. (1999). Role of p38 mitogen-
Eckle, T., Krahn, T., Grenz, A., Kohler, D., Mittelbronn, M., Lenten, C.,
Eckle, T., Hughes, K., Ehrentraut, H., Brodsky, K. S., Rosenberger, P., Choi, D. S.,
Foley, K. S., and Young, P. W. (2014). The non-muscle functions of actinin:
an update. Circulation. Res. 63, 1–34. doi: 10.1124/01.Hyp.110.032885
Fredholm, B. B., Ijzerman, A. P., Jacobson, K. A., Linden, J., and
Fredholm, B. B., Ijzerman, A. P., Jacobson, K. A., Klotz, K. N., and Linden,
(2001a). International union of pharmacology. XXII. Nomenclature, V.
Fredholm, B. B., Ijzerman, A. P., Jacobson, K. A., and Biaggioni, I. (2001).
Fredholm, B. B., Ijzerman, A. P., Jacobson, K. A., and Biaggioni, I. (2001).
Fredholm, B. B., Ijzerman, A. P., and Biaggioni, I. (2001). Comparison of
Fredholm, B. B., Irenius, E., Kull, B., and Schulte, G. (2001b). Adenosine
Fredholm, B. B., Johansson, S., and Wang, Y. Q. (2011b). Adenosine
Fuentes, E., and Palomo, I. (2015). Extracellular ATP metabolism on vascular
Darashchonak, N., Koepsell, B., Bogdanova, N., and von Versen-Hoynek, F.
Dunwiddie, T. V., Diao, L., and Proctor, W. R. (1997). Adenine nucleotides
Darashchonak, N., Koepsell, B., Bogdanova, N., and von Versen-Hoynek, F.
Dunwiddie, T. V., Diao, L., and Proctor, W. R. (1997). Adenine nucleotides
Darashchonak, N., Koepsell, B., Bogdanova, N., and von Versen-Hoynek, F.
Dunwiddie, T. V., Diao, L., and Proctor, W. R. (1997). Adenine nucleotides
Darashchonak, N., Koepsell, B., Bogdanova, N., and von Versen-Hoynek, F.
Dunwiddie, T. V., Diao, L., and Proctor, W. R. (1997). Adenine nucleotides
Darashchonak, N., Koepsell, B., Bogdanova, N., and von Versen-Hoynek, F.
Dunwiddie, T. V., Diao, L., and Proctor, W. R. (1997). Adenine nucleotides
Darashchonak, N., Koepsell, B., Bogdanova, N., and von Versen-Hoynek, F.
Dunwiddie, T. V., Diao, L., and Proctor, W. R. (1997). Adenine nucleotides
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Adenosine A2B Receptor

Hua, X., Kovarova, M., Chason, K. D., Nguyen, M., Koller, B. H., and Tilley, S. L. (2007). Enhanced mast cell activation in mice deficient in the A2B adenosine receptor. J. Exp. Med. 204, 117–128. doi: 10.1084/jem.20061572

Huang, P., Lazarowski, E. R., Tarran, R., Milgram, S. L., Boucher, R. C., and Stutts, M. J. (2001). Compartmentalized autocrine signaling to cystic fibrosis transmembrane conductance regulator at the apical membrane of airway epithelial cells. Proc. Natl. Acad. Sci. U.S.A. 98, 14120–14125. doi: 10.1073/pnas.241318498

Jacobson, K. A., and Gao, Z. G. (2006). Adenosine receptors as therapeutic targets. Nat. Rev. Drug Discov. 5, 247–264. doi: 10.1038/nrd1983

Jimenez, A. I., Castro, E., Mirabet, M., Franco, R., Delgado, E. G., and Miras-Portugal, M. T. (1999). Potentiation of ATP calcium responses by A2B receptor stimulation and other signals coupled to Gs proteins in type-1 cerebellar astrocytes. Glia 26, 119–128.

Johnston-Cox, H., Eisenstein, A. S., Koupenova, M., Carroll, S., and Ravid, K. (2014). The macrophage A2B adenosine receptor regulates tissue inflammation. PLoS ONE 9:e87775. doi: 10.1371/journal.pone.0087775

Johnston-Cox, H., Koupenova, M., Yang, D., Corkey, B., Gokce, N., Farb, M. G., Kowal, J. M., Yegutkin, G. G., and Novak, I. (2015). ATP release, generation and metabolism. Proc. Natl. Acad. Sci. U.S.A. 112(Pt 4), 491–502.

Merighi, S., Simioni, C., Gessi, S., Varani, K., Miranda, P., Tabrizi, M. A., et al. (2009). A2B and A3 adenosine receptors modulate vascular endothelial growth factor and interleukin-8 expression in human melanoma cells treated with etoposide and doxorubicin. Neoplasia 11, 1064–1073. doi: 10.1016/j.neo.099768

Mirabet, M., Herrera, C., Cordero, O. J., Mallo, J., Lluis, C., and Franco, R. (1999). Expression of A2B adenosine receptors in human lymphocytes: their role in T cell activation. J. Cell Sci. 112(Pt 4), 491–502.

Morello, S., and Miele, L. (2014). Targeting the adenosine A2B receptor in the tumor microenvironment overcomes local immunosuppression by myeloid-derived suppressor cells. Oncotarget 3, 23799–23809. doi: 10.1016/j.onco.2014.09.001

Moriyama, K., and Sitkovsky, M. V. (2010). Adenosine A2A receptor is involved in cell surface expression of A2B receptor. J. Biol. Chem. 285, 39271–39288. doi: 10.1074/jbc.M110.098239

Mundell, S. J., Mathara, A. L., Kelly, E., and Benovic, J. L. (2000). Arrestin isoforms dictate differential kinetics of A2A adenosine receptor trafficking. Biochemistry 39, 12828–12836. doi: 10.1021/bi0010928

Murakami, S., Terakura, M., Katamani, T., Hashikawa, T., Saho, T., Shimabukuro, Y., et al. (2000). Adenosine regulates the production of interleukin-6 by human gingival fibroblasts via cyclic AMP/protein kinase A pathway. J. Periodont. Res. 35, 93–101. doi: 10.1036/1569-0757.2000.0350209.x

Nehring, R. B., Horikawa, H. P., El Far, O., Kneussl, M., Brandstatter, H. J., Stamm, S., et al. (2000). The metabotropic GABAB receptor directly interacts with the activating transcription factor 4. J. Biol. Chem. 275, 35185–35191. doi: 10.1074/jbc.m002722052

Nemeth, Z. H., Bleich, D., Csoka, B., Pacher, P., Mahley, J. G., Himer, L., et al. (2007). Adenosine receptor activation ameliorates type 1 diabetes. FASEB J. 21, 2379–2388. doi: 10.1096/fj.07-8213com

Nemeth, Z. H., Leibovich, S. J., Deitch, E. A., Vizi, E. S., Szabo, C., and Hasko, G. (2003). cDNA microarray analysis reveals a nuclear factor-kappaB-independent regulation of macrophage function by adenosine. J. Pharmacol. Exp. Ther. 306, 1042–1049. doi: 10.1124/jpet.103.032944

Nguyen, D. K., Montesinos, M. C., Williams, A. J., Kelly, M., and Cronstein, B. N. (2003). Th1 cytokines regulate adenosine receptors and their downstream signaling elements in human microvascular endothelial cells. J. Immunol. 171, 3991–3998. doi: 10.4049/jimmunol.171.8.3991

Nishida, K., Doi, H., Yamanaka, M., Miyata, A., Tsukamato, K., Yabu, M., et al. (2014). Expression of adenosine A2B receptor in rat type II and III taste cells. Histochem. Cell Biol. 141, 499–506. doi: 10.1007/s00418-013-1171-0

Novitskii, S. V., Ryzhou, S., Zaynagetidinov, R., Goldstein, A. E., Huang, Y., Tikhomirov, O. Y., et al. (2008). Adenosine receptors in regulation of dendritic cell differentiation and function. Blood 112, 1822–1831. doi: 10.1182/blood-2008-02-136325

Ntantine, E., Gonyo, P., Lorimer, E. L., Hauser, A. D., Schul, N., McAllister, D., et al. (2013). An adenosine-mediated signaling pathway suppresses prenylation of the GTPase Rap1B and promotes cell scattering. Sci. Signal. 6, ra39. doi: 10.1126/scisignal.2003374

Ohta, A., and Sitkovsky, M. (2014). Extracellular adenosine-mediated modulation of regulatory T cells. Front. Immunol. 5:304. doi: 10.3389/fimmu.2014.00304

Oikonomou, K. G., Zachou, K., and Dalekos, G. N. (2011). Alpha-actinin: a multidisciplinary protein with important role in B-cell driven autoimmunity. Autoimmun. Rev. 10, 389–396. doi: 10.1016/j.autrev.2010.12.009

Pacheco, R., Martinez-Navio, J. M., Lejeune, M., Climent, N., Oliva, H., Gatell, J. M., et al. (2005). CD26, adenosine deaminase, and adenosine receptors mediate costimulatory signals in the immunological synapse. Proc. Natl. Acad. Sci. U.S.A. 102, 9583–9588. doi: 10.1073/pnas.0501050102

Panjehpour, M., Castro, M., and Klotz, K. N. (2005). Human breast cancer cell line MDA-MB-231 expresses endogenous A2B adenosine receptors mediating a Ca2+-signal. Br. J. Pharmacol. 145, 211–218. doi: 10.1038/sj.bjp.0706180

Patel, L., and Thaker, A. (2014). The effects of adenosine A2B receptor inhibition on VEGF and nitric oxide-activated renal function in diabetic nephropathy. Ren. Fail. 36, 916–924. doi: 10.3109/0886022X.2014.900404

Pawlak, N., Wu, W., Mishra, P. K., Chen, F., Millman, A., Csoka, B., et al. (2014). A2B adenosine receptor induces protective antihelmintic type 2 immune responses. Cell Host Microbe 15, 339–350. doi: 10.1016/j.chom.2014.02.001

Peckman, M. C., and Hill, S. J. (1994). Adenosine A2B-receptor-mediated cyclic AMP accumulation in primary rat astrocytes. Br. J. Pharmacol. 111, 191–198. doi: 10.1111/j.1476-5381.1994.tb14033.x
Adenosine A_{2B} Receptor

Wei, Q., Costanzi, S., Balasubramanian, R., Gao, Z. G., and Jacobson, K. A. (2013). A_{2B} adenosine receptor blockade inhibits growth of prostate cancer cells. *Purinergic Signal.* 9, 271–280. doi: 10.1007/s11302-012-9350-3

Wen, J., Wang, B., Du, C., Xu, G., Zhang, Z., Li, Y., et al. (2015). A_{2B} Adenosine receptor agonist improves erectile function in diabetic rats. *Tohoku J. Exp. Med.* 237, 141–148. doi: 10.1620/tjem.237.141

White, J. H., Mcllhinney, R. A., Wise, A., Ciruela, F., Chan, W. Y., Emson, P. C., et al. (2000). The GABAB receptor interacts directly with the related transcription factors CREB2 and ATFx. *Proc. Natl. Acad. Sci. U.S.A.* 97, 13967–13972. doi: 10.1073/pnas.240452197

WHO. (2014). *Global Status Report on Noncommunicable Diseases 2014.* World Health Organization.

Wilson, C. N., Nadeem, A., Spina, D., Brown, R., Page, C. P., and Mustafa, S. J. (2009). Adenosine receptors and asthma. *Handb. Exp. Pharmacol.* 193, 329–362. doi: 10.1007/978-3-540-89615-9_1

Xaus, J., Mirabet, J., Lloberas, J., Soler, C., Lluis, C., Franco, R., et al. (1999). IFN-gamma up-regulates the A_{2B} adenosine receptor expression in macrophages: a mechanism of macrophage deactivation. *J. Immunol.* 162, 3607–3614.

Yaar, R., Jones, M. R., Chen, J. F., and Ravid, K. (2005). Animal models for the study of adenosine receptor function. *J. Cell. Physiol.* 202, 9–20. doi: 10.1002/jcp.20138

Yang, D., Chen, H., Koupenova, M., Carroll, S. H., Eliades, A., Freedman, J. E., et al. (2010a). A new role for the A_{2B} adenosine receptor in regulating platelet function. *J. Thromb. Haemost.* 8, 817–827. doi: 10.1111/j.1538-7836.2010.03769.x

Yang, D., Koupenova, M., McCrann, D. J., Kopeikina, K. I., Kagan, H. M., Schreiber, B. M., et al. (2008). The A_{2B} adenosine receptor protects against vascular injury. *Proc. Natl. Acad. Sci. U.S.A.* 105, 792–796. doi: 10.1073/pnas.0705563105

Yang, D., Zhang, Y., Nguyen, H. G., Koupenova, M., Chauhan, A. K., Makitalo, M., et al. (2006). The A_{2B} adenosine receptor protects against inflammation and excessive vascular adhesion. *J. Clin. Invest.* 116, 1913–1923. doi: 10.1172/JCI27933

Yang, M., Ma, C., Liu, S., Shao, Q., Gao, W., Song, B., et al. (2010b). HIF-dependent induction of adenosine receptor A_{2B} skews human dendritic cells to a Th2-stimulating phenotype under hypoxia. *Immunol. Cell Biol.* 88, 165–171. doi: 10.1038/icb.2009.77

Zaynageldinov, R., Ryzhov, S., Goldstein, A. E., Yin, H., Novitskiy, S. V., Goleniewska, K., et al. (2010). Attenuation of chronic pulmonary inflammation in A_{2B} adenosine receptor knockout mice. *Am. J. Respir. Cell Mol. Biol.* 42, 564–571. doi: 10.1165/rcmb.2008-0391OC

Zhang, W., Zhang, Y., Wang, W., Dai, Y., Ning, C., Luo, R., et al. (2013). Elevated ecto-5’-nucleotidase-mediated increased renal adenosine signaling via A_{2B} adenosine receptor contributes to chronic hypertension. *Circ. Res.* 112, 1466–1478. doi: 10.1161/CIRCRESAHA.111.300166

Zhao, X., Hsu, K. S., Lim, J. H., Bruggeman, L. A., and Kao, H. Y. (2015). alpha-Actinin 4 potentiates nuclear factor kappa-light-chain-enhancer of activated B-cell (NF-kappaB) activity in podocytes independent of its cytoplasmic actin binding function. *J. Biol. Chem.* 290, 338–349. doi: 10.1074/jbc.M114.597260

Zhou, Y., Mohsenin, A., Morschel, E., Young, H. W., Molina, J. G., Ma, W., et al. (2009). Enhanced airway inflammation and remodeling in adenosine deaminase-deficient mice lacking the A_{2B} adenosine receptor. *J. Immunol.* 182, 8037–8046. doi: 10.4049/jimmunol.0900515

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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