Adenosine Promotion of Cellular Migration in Bronchial Epithelial Cells Is Mediated by the Activation of Cyclic Adenosine Monophosphate–Dependent Protein Kinase A

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Migration of neighboring cells into the injury is important for rapid repair of damaged airway epithelium. We previously reported that activation of the A_{2A} receptors (A_{2A}ARs) mediates adenosine-stimulated epithelial wound healing, suggesting a role for adenosine in migration. Because A_{2A}AR increases cyclic adenosine monophosphate (cAMP) levels in many cells, we hypothesized that cAMP-dependent protein kinase A (PKA) is involved in adenosine-mediated cellular migration.

To test this hypothesis, we stimulated a human bronchial epithelial cell line with adenosine and/or A_{2A}AR agonist (5′-(N-cyclopropyl)-carboxamido-adenosine [CPCA]) in the presence or absence of adenosine deaminase inhibitor (erythro-9-(2-hydroxy-3-nonyl) adenine hydrochloride [EHNA]).

Cells treated with adenosine or CPCA demonstrated a concentration-dependent increase in migration. Similar results were observed in the presence and absence of EHNA. To confirm A_{2A} involvement, we pretreated the cells for 1 hour with the A_{2A} receptor antagonist ZM241385 and then stimulated them with either adenosine or CPCA. To elucidate PKA's role, cells were pretreated for 1 hour with either a PKA inhibitor (KT5720) or a cAMP antagonist analogue (Rp-cAMPS) and then stimulated with adenosine and/or CPCA. Pretreatment with KT5720 or Rp-cAMPS resulted in a significant decrease in adenosine-mediated cellular migration. PKA activity confirmed that bronchial epithelial migration requires cAMP and PKA activity. When cells were wounded and stimulated with CPCA, an increase in PKA activity occurred. Pretreatment for 1 hour with either KT5720 or Rp-cAMPS resulted in a significant decrease in adenosine-mediated PKA activation.

These data suggest that adenosine activation of A_{2A}AR augments epithelial repair by increasing airway cellular migration by PKA-dependent mechanisms.

Key words: adenosine, airway repair, cellular migration

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Repair of injured airway is accomplished by migration of epithelial cells from the edge of a wound and restitution of the epithelial barrier. We and others have demonstrated that a number of stimuli are capable of augmenting bronchial epithelial cell migration in Boyden blind-well chamber migration assays.[1–4] It is clear that a variety of stimuli potentially modulate the ability of bronchial epithelial cells to disengage from their normal cell-cell and cell-matrix relationships and move into a wound area.[1,3–5] Adenosine, a purine nucleoside known to modulate hypoxic, ischemic, and inflammatory processes critical to both tissue homeostasis and injury through specific cell surface receptors,[6–8] has recently been investigated for its impact on the cells and tissues of healing wounds.

We recently demonstrated that activation of the A_{2A} receptors (A_{2A}ARs) mediates epithelial wound healing in bronchial epithelial cells,[9] and this effect was apparent as early as 2 hours after wounding. Proliferation, migration, and synthesis of message for angiogenic growth factor are some of the numerous adenosine-mediated effects that may contribute to the accelerated rate of wound closure.[10,11] Other studies have demonstrated, depending on the size of the wound, that cells will flatten and migrate to cover the open area long before proliferation becomes important.[12,13] We can conclude from these studies that for wound closure to be accelerated early in the healing process, adenosine acting at A_{2A}AR must stimulate either cell spreading or cell migration and that a common pathway for controlling cell movement might be influenced by cyclic nucleotides and cyclic nucleotide–dependent kinases.

Cyclic nucleotides such as cyclic adenosine monophosphate (cAMP) are known to modulate cell shape and attachment as well as movements of some cells.[14,15] Upon activation of adenylyl cyclase, cAMP is synthesized, and the major downstream effector, cAMP-dependent protein kinase A (PKA), is activated. PKA has been well described in airway epithelial cells,[16,17] and evidence suggests that cAMP levels and PKA modulate the migration of some tumor cell lines, epidermal cells, endothelial cells, and bronchial epithelial cells.[18–21]

In the present study, we hypothesized that adenosine-mediated migration occurs via activation of A_{2A}AR and is influenced by cAMP and the activation of its major binding protein, PKA. To address this hypothesis, we used the human bronchial epithelial cell line (BEAS-2B) as an in vitro model of cell migration using a 48-well Boyden chamber. We investigated the role of adenosine in cellular migration and found that adenosine promotes migration via the activation of the A_{2A}AR. Blockage of the A_{2A}AR by a potent A_{2A} receptor antagonist, ZM241385, inhibited cellular migration. The addition of a cAMP antagonist or PKA inhibitor was used to confirm a role for PKA activity in adenosine-stimulated bronchial epithelial cell migration. Our findings will provide a better understanding of the roles and mechanisms underlying adenosine-mediated cellular migration as it relates to airway injury and repair processes.

Materials and Methods

Reagents/Materials

LHC (Laboratory of Human Carcinogenesis) basal medium and M199 medium were purchased from Biofluids (Rockville, MD). RPMI 1640 (Roswell Park Memorial Institute), streptomycin, penicillin, protease (type IV), fetal calf serum, and fungizone were purchased from Invitrogen (Carlsbad, CA). The type I collagen gel matrix Vitrogen 100 was purchased from Cohesion (Palo Alto, CA). ZM241385 (A_{2A} receptor antagonist) was purchased from Tocris (Ellisville, MO). Bovine plasma fibronectin was purchased from Calbiochem-Novabiochem (San Diego, CA). KT-5720 (PKA inhibitor) and Rp-cAMPS (cAMP antagonist analogue) were purchased from Alexis Biochemical (San Diego, CA).[22] The CAMP immunoassay kit was purchased from R&D Systems (Minneapolis, MN). Adenosine, CPCA (5′-(N-cyclopropyl)-carboxamido-adenosine; A_{2A} receptor agonist), EHNA (erythro-9-(2-hydroxy-3-nonyl) adenine hydrochloride; adenosine deaminase inhibitor), and all other reagents not listed were purchased from Sigma (St. Louis, MO).

Cell Culture

The immortalized human BEAS-2B bronchial epithelial cell line was purchased from the American Type Culture Collection (Rockville, MD). Cells were cultured on type I collagen– (Vitrogen 100) coated dishes in serum-free medium, LHC-9/RPMI.[23] The usual LHC9 medium contains epinephrine (0.5 μg/mL) and retinoic acid (0.33 μmol), and this was used for initial growth of the cells. Cells were placed in fresh medium without epinephrine and retinoic acid the night before all migration experiments (LHC-D).

Boyden Chamber Bronchial Epithelial Cell Migration

Confluent BEAS-2B cells were detached by LHC-D containing 0.05% trypsin and 0.53 mmol ethylenediaminetetraacetic acid and washed with LHC-D containing soybean trypsin inhibitor (2 mg/mL; Sigma) to neutralize the trypsin. Cells were suspended in LHC-D at 1×10^6 cells/mL and used for migration assay with the Boyden chamber technique using a 48-well multiwell chamber (Neuroprobe, Bethesda, MD).[1] Polycarbonate membranes with 8 μm pores (Neuroprobe) were coated with 0.1% gelatin (Bio-Rad, Richmond, CA), as previously described.[1] Fibronectin (30 μg/mL) was used in the bottom wells as an chemoattractant.[24–26] The BEAS-2B cells were pretreated for 1 hour with or without the inhibitor of adenosine deaminase (EHNA; 10 μmol), cAMP antagonist (Rp-cAMPS; 5 μmol), or PKA inhibitor (KT5720; 1 μmol), stimulated with several concentrations of either adenosine or CPCA (1 nM–100 μmol) and then placed into each of the top wells above the filter. The chambers were then incubated at 37°C, 5% CO_2 for 5 hours. After

incubation, cells on the top of the filter were removed by scraping. The filter was then stained with a modified Wright stain (Diff-Quik, Dade Behring, Deerfield, IL). Epithelial cell migration activity was quantified as the number of migrated cells on the lower surface of the filter in 20 high-power fields (HPFs) using a light microscope at 400×.

Determination of cAMP Activity

cAMP activity was determined using an cAMP immunoassay kit (R&D Systems, Minneapolis, MN). BEAS-2B cells were treated with either 100 nmol adenosine or CPCA for 30 minutes. Positive control groups were treated with forskolin 100 μmol (cAMP activator) for 30 minutes. Cells were lysed and centrifuged at 600*g* for 10 minutes at 4°C, and supernates were assayed as described by the manufacturer. All samples were assayed in triplicate, and no fewer than three separate experiments ($n = 9$) were performed per unique parameter. Samples were read at a wavelength of 450 nm using a Bio-Rad Benchmark microplate reader (Bio-Rad Laboratories, Hercules, CA). Data were analyzed for significance using one-way analysis of variance (ANOVA) followed by a Tukey multiple-comparison test. Significance was assigned at $p \leq .05$.

Determination of cAMP-Dependent PKA

PKA activity was determined in crude whole-cell fractions of BEAS-2B cells. The assay employed is a modification of procedures previously described,[27] with 130 μM PKA substrate heptapeptide (LRRASLG), 10 μmol adenosine triphosphate (ATP), 0.2 mmol IBMX, 20 mmol Mg-acetate, and 0.2 mmol [γ-^{32}P] ATP in a 40 mmol Tris-HCl buffer (pH 7.5). Samples (20 μL) were added to 50 μL of the above reaction mixture and incubated for 15 minutes at 30°C. Reactions were initiated by the addition of 10 μL of cell fraction. Incubations were halted by spotting 50 μL of each sample onto P-81 phosphocellulose papers. Papers were then washed five times for 5 minutes each in phosphoric acid (75 mmol), washed once in ethanol, dried, and counted in nonaqueous scintillant, as previously described.[28] The negative control group consisted of similar assay conditions with or without the appropriate substrate peptide. The positive control group contained 10 μmol cAMP. Kinase activity is expressed in relation to total cellular protein assayed and was calculated in picomoles phosphate incorporated per minute per milligram of total protein. All samples were assayed in triplicate, and no fewer than three separate experiments ($n = 9$) were performed per unique parameter. Data were analyzed for significance using one-way ANOVA followed by a Tukey multiple-comparison test. Significance was assigned at $p \leq .05$.

Statistical Analysis

The Boyden chamber assays (48-well format) were performed in triplicate (in separate wells) and repeated in three separate experiments with similar results ($n = 3$). The data represent means ± standard error for these triplicates. Data were analyzed for significance using one-way ANOVA followed by a Tukey multiple-comparison test. Significance was assigned at $p \leq .05$.

Results

Adenosine Promotes Cellular Migration in BEAS-2B Cells

Understanding that our in vitro wound closure assay is based on migration and not proliferation, we further investigated the role of adenosine in cellular migration using the Boyden migration assay. Adenosine alone had a small effect on migration. Adenosine (1 nmol to 100 μmol) increased cells migrated per 20× HPFs from 9 ± 1.52 for control to 13 ± 0.882 for adenosine-treated cells ($p > .05$). The presence of adenosine in the lower, upper, or both wells during the assay had no effect, suggesting a small chemokinetic effect on migration. When cells were pretreated for 1 hour with an adenosine deaminase inhibitor, EHNA (10 μmol), and fibronectin (30 μg/mL) was present in the lower chambers to provide a gradient of protein for migration, adenosine had a more pronounced effect on migration (Figure 1). Likewise, cells stimulated for 5 hours with CPCA, an A_{2A}AR agonist, migrated in a concentration-dependent manner (Figure 2). To verify A_{2A}AR involvement in promoting adenosine-mediated cellular migration, cells pretreated for 1 hour with ZM241385 (100 nM; a known A_{2A} receptor antagonist) blocked adenosine's cellular migration stimulatory effect (Figure 3). Collectively, the data suggest that adenosine via occupancy of its A_{2A}AR promotes cellular migration in BEAS-2B cells.

Activation of PKA Promotes Adenosine-Mediated Migration

We previously reported that beta-agonist stimulation of PKA accelerated bronchial epithelial cell migration.[21] To evaluate if adenosine-stimulated migration activates PKA, BEAS-2B cells were pretreated with EHNA (10 μmol) and/or a PKA inhibitor, KT5720 (1 μmol), for 1 hour and then stimulated for 5 hours with either 0.1 μmol adenosine

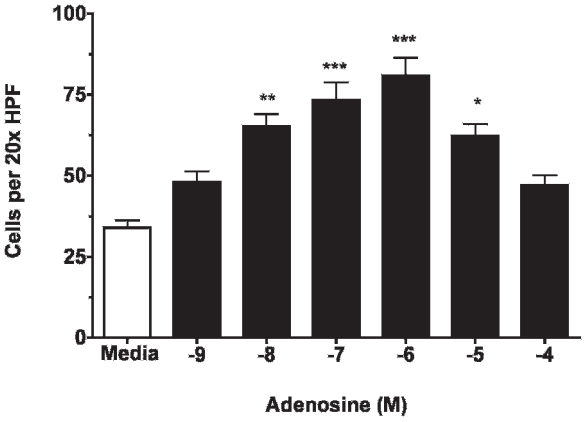

Figure 1 Adenosine-induced migration in bronchial epithelial cells is concentration dependent. BEAS-2B cells were grown to confluency, trypsinized, and used in the chamber migration assay. All cells were pretreated for 1 hour with EHNA (10 μmol) before stimulation with adenosine. Adenosine at the concentrations shown was added to the cells in the upper wells. All conditions had 30 μg/mL of fibronectin present in the lower wells as an attractant. The figure is representative of three independent experiments performed in triplicate ± standard error of measurement, $n = 3$ ($*p < .05$; $**p < .01$; $***p < .001$ vs media control). HPF = high-power field.

or CPCA. Cells stimulated alone with adenosine or CPCA revealed a significant increase in cellular migration compared with media control (Figure 4). Cells pretreated with KT5720 and stimulated with

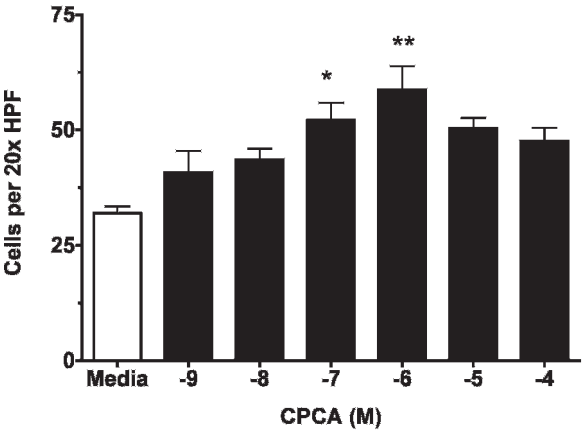

Figure 2 Occupancy of the A_{2A} receptor promotes migration in BEAS-2B cells. Bronchial epithelial cells were prepared and assayed for migration as described above. Fibronectin (30 μg/mL) was present in the lower wells as an attractant. The figure is representative of three independent experiments performed in triplicate ± standard error of measurement, $n = 3$ ($*p < .05$; $**p < .01$ vs media control).

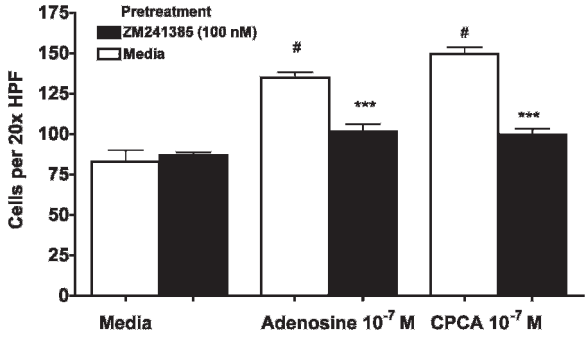

Figure 3 ZM241385, an A_{2A} receptor antagonist, blocks adenosine-mediated cellular migration in BEAS-2B cells. To confirm a role for A_{2A} receptor involvement in adenosine-mediated migration, cells were pretreated for 1 hour with or without an antagonist of A_{2A}AR, ZM241385. Adenosine (100 nmol) or CPCA (an A_{2A} receptor agonist; 100 nmol) was added to the cells in the upper wells. All conditions had 30 μg/mL of fibronectin present in the lower wells as an attractant. The figure is representative of three independent experiments performed in triplicate ± standard error of measurement, $n = 3$ ($^{\#}p < .01$ vs media control; $***p < .001$ vs adenosine-treated control).

adenosine or CPCA revealed that KT5720 blocked the adenosine-mediated cellular stimulatory effect. These findings suggest that activation of PKA is essential for A_{2A}AR-mediated cellular migration in BEAS-2B cells.

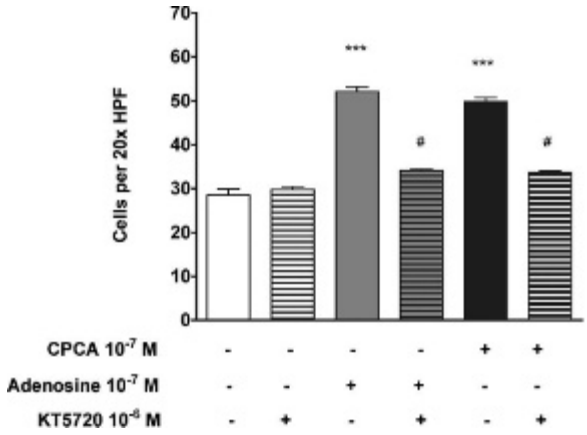

Figure 4 Inhibition of protein kinase A (PKA) blocks adenosine-mediated migration. BEAS-2B cells were pretreated for 1 hour with or without a PKA inhibitor, KT5720 (1 μmol). Adenosine or CPCA (100 nmol) was added to the cells in the upper wells. All conditions had 30 μg/mL of fibronectin present in the lower wells as an attractant. The figure is representative of three independent experiments performed in triplicate ± standard error of measurement, $n = 3$ ($***p < .001$ vs media control; $^{\#}p < .001$ vs adenosine-treated cells).

Rp-cAMPS, a cAMP Antagonist Analogue, Blocks A_{2A}AR-Mediated Cellular Migration in Bronchial Epithelial Cells

To confirm whether A_{2A}AR-mediated cellular migration is dependent on adenosine-mediated cAMP activation of PKA, BEAS-2B cells were pretreated with EHNA (10 μmol) and/or a specific cAMP antagonist, Rp-cAMPS (5 μmol), for 1 hour and then stimulated for 5 hours with either 0.1 μmol adenosine or CPCA. Cells stimulated alone with adenosine or CPCA revealed a significant increase in cellular migration compared with media control (Figure 5). Cells pretreated with Rp-cAMPS and stimulated with adenosine or CPCA revealed that Rp-cAMPS significantly decreased adenosine-mediated cellular migration. The data confirmed that adenosine promotion of cellular migration is dependent on the synthesis of cAMP and subsequent activation of PKA.

Adenosine Receptor Activation Stimulates cAMP Activity in BEAS-2B Cells

It has been documented that adenosine receptors have the ability to induce cAMP-dependent processes in many cell types.[29,30] To authenticate occupancy of A_{2A}AR-mediated accumulation of cAMP, BEAS-2B cells were stimulated with 0.1 μmol adenosine or CPCA for 30 minutes. In some dishes, the cells were treated with a cAMP activator, forskolin (100 μmol), as a positive control to allow accumulation of cAMP. Adenosine or CPCA stimulated a ≈2-fold increase in cAMP accumulation compared with media controls, and positive control cells had a ≈4-fold increase in cAMP accumulation (Figure 6). These data confirmed that adenosine occupancy of the A_{2A} receptor(s) stimulates intracellular accumulation of cAMP in BEAS-2B cells and further substantiate that adenosine-mediated cellular migration induces cAMP-dependent activation of PKA.

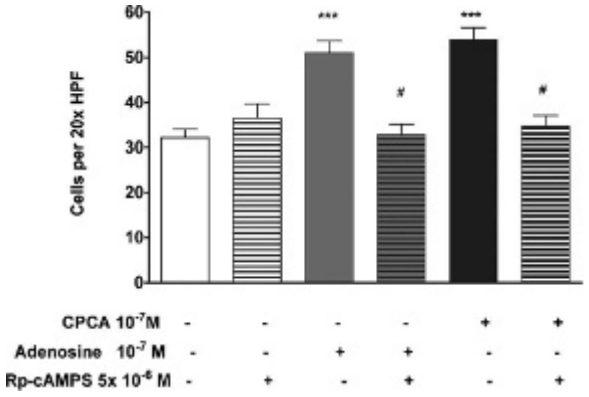

Figure 5 Rp-cAMPS blocks A_{2A}-mediated cellular migration in BEAS-2B cells. BEAS-2B cells were pretreated with a specific cAMP inhibitor, Rp-cAMPS (5 μmol), for 1 hour and then stimulated with the indicated concentration of adenosine or CPCA in the upper wells. All conditions had 30 μg/mL of fibronectin present in the lower wells as an attractant. The figure is representative of three independent experiments performed in triplicate ± standard error of measurement, $n = 3$ (***$p < .001$ vs media control; $^{\#}p < .001$ vs adenosine-treated cells).

Rp-cAMPS and KT5720 Blocks A_{2A}AR-Mediated PKA Activation in Wounded BEAS-2B Cells

Because the in vitro wound closure assay used is virtually all cell spreading and migration[9,31] and intracellular kinases are critical for wound healing to occur, BEAS-2B PKA activity was measured in response to adenosine in the presence and absence of a cAMPS antagonist analogue and a PKA inhibitor. BEAS-2B cells were wounded as described,[9] and all cells were pretreated with IBMX (200 μmol; phosphodiesterase inhibitor) and EHNA (10 μmol) with or without Rp-cAMPS (5 μmol) or KT5720 (1 μmol) for 1 hour followed by stimulation with CPCA (100 nmol) for 30 minutes. Wounded cells stimulated alone with CPCA revealed a significant increase in PKA activity compared with media control (Figure 7).

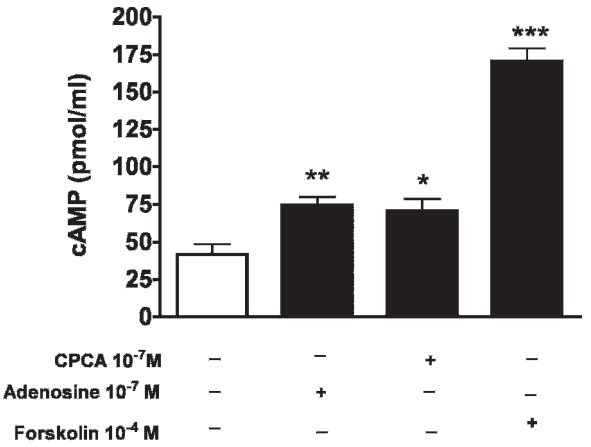

Figure 6 Occupancy of the A_{2A} receptor stimulates cAMP accumulation in BEAS-2B cells. Confluent monolayers of BEAS-2B cells were stimulated for 30 minutes with the indicated concentration of adenosine or CPCA and the positive control group was treated with forskolin (100 μmol). Supernates from cell lysates were assayed for cAMP accumulation. Adenosine or CPCA stimulated a ≈2-fold activity increase in cAMP activity. Positive control group stimulated a ≈4-fold activity increase in cAMP activity. The figure is representative of one experiment performed in triplicate ± standard error of measurement, $n = 9$ (***$p <$ .001 vs media control; **$p < .01$ vs media control; *$p < .05$ vs media control).

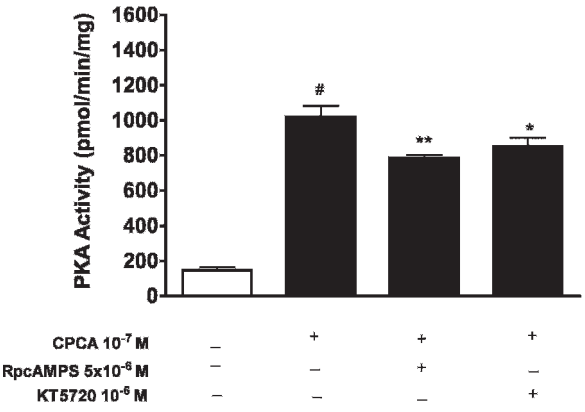

Figure 7 Rp-cAMPS and KT5720 blocks adenosine-mediated activation of protein kinase A (PKA) in BEAS-2B cells. Confluent monolayers of BEAS-2B were pretreated with either Rp-cAMPS (5 μmol) or KT5720 (1 μmol) for 1 hour, wounded, stimulated with CPCA (100 nmol) for 30 minutes, and flash-frozen in cell lysis buffer (see Materials and Methods). Cell homogenates were assayed for PKA activity. CPCA stimulated a ≈3-fold activity increase in PKA. Pretreatment with either Rp-cAMPS or KT5720 significantly reduced CPCA-mediated activation of PKA. The figure is representative of three independent experiments performed in triplicate ± standard error of measurement, $n = 9$ ($^{\#}p <$ .001 vs media control; $^{**}p <$ .01 vs CPCA treated; $^{*}p <$ .05 vs CPCA treated).

Pretreatment with either Rp-cAMPS or KT5720 significantly blocked CPCA-mediated activation of PKA in wounded BEAS-2B cells. The data demonstrate that PKA is a critical component to facilitate cellular migration in wounded bronchial epithelial cells.

Discussion

The present study demonstrated that activation of A_{2A}AR(s) by adenosine promotes cellular migration in bronchial epithelial cells via a cAMP-mediated signal transduction pathway. In addition, our studies also revealed that adenosine-mediated cellular migration is dependent on the activation of cAMP-dependent protein kinase.

Previous studies have suggested that adenosine acting at the A_{2A}AR stimulates endothelial cell migration.[2,32] Cellular migration is an essential early event in the wound healing process. Many stimuli have been reported to modulate cellular migration in airway epithelium.[24,33–35] Our data indicate that in the presence of fibronectin, adenosine-stimulated cellular migration increased in response to this chemoattractant, and this was mediated by the activation of PKA. A similar response was also observed when cells were stimulated with CPCA, an A_{2A}AR agonist. Moreover, ZM-241385, a potent and highly selective A_{2A} adenosine antagonist with a low potency at the A_{2B} receptor,[36,37] further reinforce that A_{2A}AR elicits migration. We observed a slight decrease in cellular migration when cells were stimulated with high concentrations (10–100 μmol) of either adenosine or CPCA, suggesting the possibility of cross-activation among the other adenosine receptors (ie, A_1AR, A_{2B}AR, or A_3AR) that might result in the activation of an inhibitory kinase, that is, protein kinase C (PKC). A number of potential signaling pathways are associated with adenosine-mediated effects. Cyclic nucleotides, such as cAMP and cyclic guanosine monophosphate, are both known to modulate cell shape, attachment, and cellular movement.[14,38] Work conducted earlier in our laboratory revealed that PKC activation retards wound repair.[39] Further studies are under way to evaluate the involvement of multiple intracellular pathways that modulate adenosine-mediated cellular migration. Our finding that occupancy of the A_{2A}AR(s) contributes to the adenosine-stimulatory migratory effect was not surprising. We previously demonstrated that adenosine accelerates wound closure via occupancy of the A_{2A}AR(s), whereas occupancy of either the A_1AR or A_3AR slows wound closure. Occupancy of A_{2B}AR has little to no effect on wound closure.[9] The next logical step in investigating adenosine's role in modulating wound healing is to look at the early events critical for wound repair. The data indirectly indicate that adenosine has the ability to disengage the cell-cell and cell-matrix relationship, allowing the cells to move into a wound area. These mechanisms are dependent on the extracellular matrix, which forms the support for the cells during wound repair. Supplementary studies are ongoing to determine adenosine's role in modulating cell surface adhesion molecules (ie, integrins) that are known to be involved in the regulation of cell migration and proliferation.[40]

To gain further insight into how adenosine stimulates migration, we investigated the signal transduction pathway involved. It has been well documented that A_{2A}AR(s) proceeds at least in part via activation of G protein leading to cAMP signaling events and activation of PKA.[41,42] We have demonstrated that the cAMP analogue dibutyryl cAMP used to directly activate PKA stimulated fibronectin-induced bovine bronchial epithelial cell migration.[21] Our previous studies suggested that cellular migration in response to wounding is regulated by agents that elevate intracellular cAMP levels, subsequently activat-

ing PKA.[21,31] However, little is known about adenosine-induced cellular migration. Our data revealed that Rp-cAMPS, a cAMP antagonist analogue, and KT5720, a PKA inhibitor, significantly reduced adenosine-stimulated cellular migration. Likewise, we demonstrated that adenosine-mediated occupancy of A_{2A}AR(s) in human bronchial epithelial cells increased intracellular cAMP accumulation. As confirmation, PKA activity was assessed to demonstrate the efficacy of these pharmacologic tools in wounded bronchial epithelial cells pretreated with either Rp-cAMPS or KT5720. The data also revealed a significant reduction in adenosine-mediated PKA activation. In contrast to our findings, Zhong and colleagues reported that A_{2A} receptor occupancy by CGS-21680, an A_{2A} selective agonist, did not cause a significant increase in cellular cAMP.[43] Their findings were correlated with decreased messenger ribonucleic acid (mRNA) expression of A_{2A}AR. Interestingly, like their findings, under basal condition, our BEAS-2B cells expressed relatively low mRNA expression of A_{2A} receptor; however, when cells were exposed to cigarette smoke, a model of airway injury, we observed an upregulation in mRNA expression of A_{2A}, A_{2B}, and A_3 receptors.[9] Studies conducted by Nadeem and colleagues revealed decreased expression of A_{2A} receptors in sensitized wild-type A_{2A} receptor–deficient allergic mice and absence of detectable A_{2A} receptor transcripts in their knockout mice.[44] In addition, this decrease was associated with a decrease in cAMP levels in the lung. Indeed, the fundamental differences in the mechanism of action for activation of A_{2A} receptors may depend on the use of specific A_{2A} receptor agonists, particularly CPCA versus CGS21680. Collectively, our data suggest that stimulation of cAMP and activation of its major cellular receptor, PKA, are critical for this adenosine-mediated effect on cell migration.

The ability of adenosine to stimulate bronchial epithelial cell migration in vitro supports the hypothesis that adenosine may promote proliferation in vivo. The in vitro wound closure assay we used is virtually an assay of cell spreading and migration,[9,21,31] with little cell proliferation responsible for wound closure. Several studies have also suggested that migration occurs quickly, indicating that proliferation is not so important in the early stages of wound healing.[12,13, 45] Adenosine is generated as a result of ATP catabolism and thus is well suited for the role of metabolic regulator. The physiologic and pharmacologic effects of adenosine acting at one or another receptor are observed in nearly every tissue and organ. Our previous studies revealed not only that all four adenosine receptors are present in bronchial epithelial cells but also that they become unregulated when injury occurs.[9] The studies reported here confirm that enhancement of wound healing and cellular migration is mediated by A_{2A}AR(s). The observations reported here further suggest that targeting A_{2A}AR may lead to development of agents that are useful for stimulating wound healing.

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