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REVIEW

New insights regarding the regulation of chemotaxis by nucleotides, adenosine, and their receptors

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Abstract The directional movement of cells can be regulated by ATP, certain other nucleotides (e.g., ADP, UTP), and adenosine. Such regulation occurs for cells that are "professional phagocytes" (e.g., neutrophils, macrophages, certain lymphocytes, and microglia) and that undergo directional migration and subsequent phagocytosis. Numerous other cell types (e.g., fibroblasts, endothelial cells, neurons, and keratinocytes) also change motility and migration in response to ATP, other nucleotides, and adenosine. In this article, we review how nucleotides and adenosine modulate chemotaxis and motility and highlight the importance of nucleotide- and adenosine-regulated cell migration in several cell types: neutrophils, microglia, endothelial cells, and cancer cells. We also discuss difficulties in conducting experiments and drawing conclusions regarding the ability of nucleotides and adenosine to modulate the migration of professional and non-professional phagocytes.

Keywords Regulation · Chemotaxis · Nucleotides · Adenosine · Receptors

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Introduction

Motility and migration of cells are fundamental biological properties that contribute to normal development and differentiation. These processes are particularly important in the function of "professional phagocytes," which include neutrophils, macrophages, and microglia [1–3]. Such cells have essential roles in migrating toward and engulfing microorganisms and apoptotic cells, events that depend on the detection of chemoattractants or paracrine "find-me/eat-me" signals, respectively [4, 5]. Directed migration is also important for other cell types and contributes to normal cell function (e.g., development and angiogenesis) and pathophysiological states (e.g., cancer metastasis). Evidence in recent years has indicated the importance of extracellular nucleotides and adenosine in the regulation of homeostatic mechanisms and migration of professional phagocytes and other cell types (e.g., fibroblasts, endothelial cells, keratinocytes, and neurons), including stem cells and malignant cells [6, 7].

Chemotaxis, the directed migration of cells in response to chemical cues, is characterized by three distinct processes: motility, cell polarization, and gradient sensing ([8]; Fig. 1). Cell motility, which can occur in the absence of extracellular cues [8], is driven by an actin-mediated extension of self-regulating pseudopod protrusions that facilitate random and directed cell migration. Such migration can occur in the absence of external cues as a "correlated random walk" [9], movement of cells by their sequential formation of pseudopods [10]. Such pseudopod formation is biased (i.e., occurs at one end of the cell) even in the absence of an external signal. Cell polarization involves the detection of extracellular cues and progression through a series of cytoskeletal rearrangements that promote the protrusion of a leading edge of a cell and the release of integrin-based contacts with the substratum

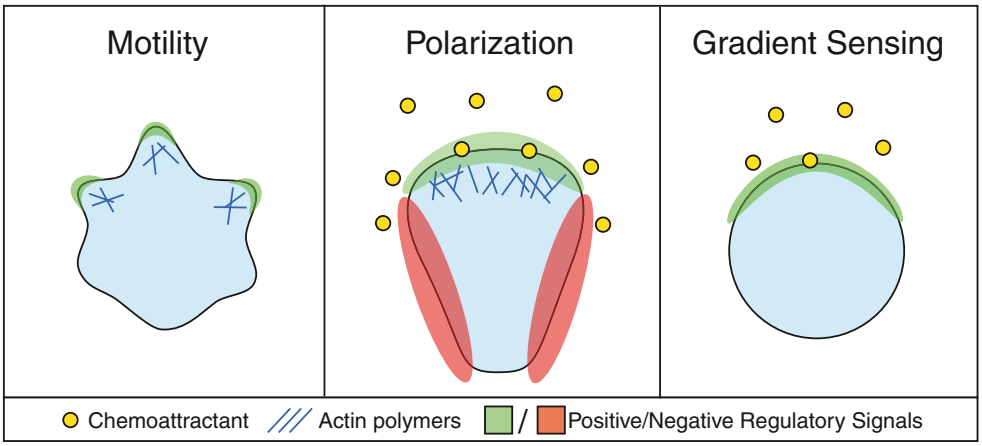

Fig. 1 Cellular events involved in chemotaxis. Cell motility is driven by the biased, actin-mediated extension of pseudopod-like extensions at one end of the cell. Some cells are capable of coordinating intracellular and extracellular signaling to promote cell polarization (*middle panel*) and establish persistent leading and trailing edges. Furthermore, even while immobile, some cells can detect and amplify external chemoattractant gradients, a process termed gradient sensing (*right panel*)

and contraction at the rear of the cell (i.e., the uropod; [11]). Cell polarization is central to the migration of many cell types.

Different cell types have varying levels of intrinsic polarity. Certain cell types exposed to a uniform concentration of chemoattractant do not polarize, but instead undergo chemokinesis (i.e., random cell migration; [11]). However, some types of cells (e.g., neutrophils and the slime mold *Dictyostelium discoidium*) can polarize under such conditions; such cell types can detect and amplify spatial gradients even while immobile, a process called gradient sensing [8].

The release of ATP and other nucleotides (e.g., UTP; or adenosine 3′,5′ cyclic AMP [cAMP] by *D. discoidium*) in response to extracellular stimuli is critical for different aspects of the chemotactic response and involves mechanisms that are discussed elsewhere in this issue by Lazarowski [12]. Extracellular ATP promotes cell migration through both autocrine and paracrine pathways. For example, professional phagocytes (e.g., neutrophils; Fig. 2) release ATP in response to stimulation with some chemoattractants. This autocrine release of ATP is critical for the chemotactic response to the chemoattractants and is discussed below. ATP released from

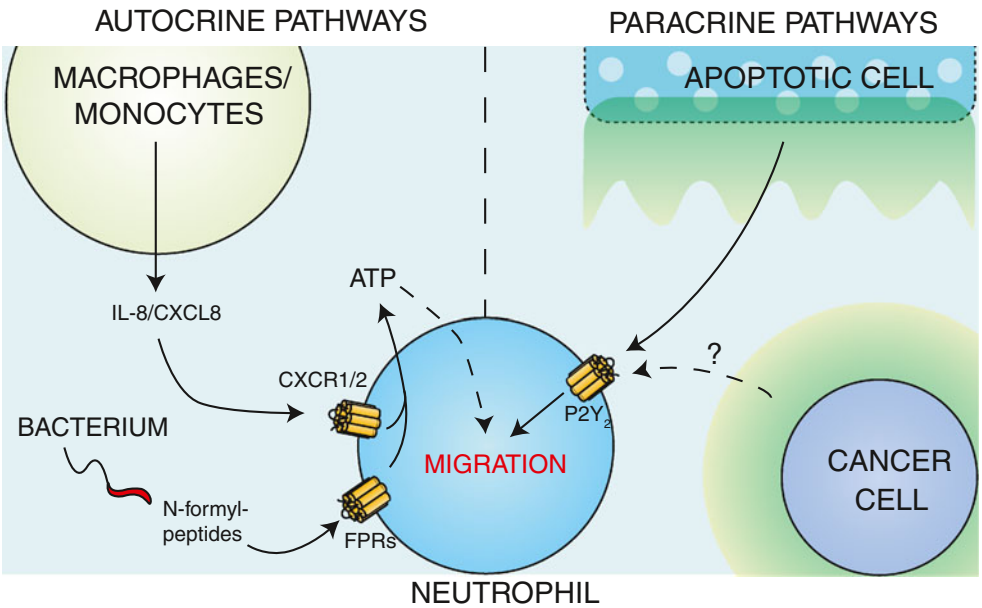

Fig. 2 Autocrine/paracrine stimulation of chemotaxis. Extracellular nucleotides promote cell migration through autocrine and paracrine pathways. In the case of neutrophils, stimulation with some chemoattractants, such as IL-8 from host cells (e.g., macrophages) or formyl peptides from damaged cells or bacteria, results in a release of ATP. Through various autocrine signaling pathways, released nucleotides promote cell migration. Alternatively, the direct release of ATP from apoptotic cells has been proposed to act as a paracrine "find me" signal to promote phagocytic clearance. Elevated levels of extracellular ATP in tumor microenvironments may similarly mediate neutrophil migration

apoptotic cells may act as a paracrine "find-me" signal that promotes phagocytic clearance [5]. There is debate as to whether ATP is a chemoattractant (see [13]) or if it promotes random cell migration (i.e., chemokinesis), acting as a local "touch-me" signal rather than a long-range "find-me" signal [14]. It is tempting to postulate that similar mechanisms may also recruit immune cells to cancerous tissues.

Released ATP can undergo numerous fates. Cells utilize multiple mechanisms to generate and respond to extracellular ATP and adenosine (Fig. 3). These include an interaction of nucleotides with one or more P2X (ion channel; [15]) and P2Y (G-protein-coupled; [16]) receptors and the enzymatic hydrolysis of ATP (e.g., via the sequential actions of NTPDases and CD73/ecto-5′-nucleotidase; [17]) to generate adenosine. Extracellular adenosine, in turn, can interact with one or more G-protein-coupled P1 receptors [18] or can be taken back up into cells via ENT (equilibrative nucleoside transporter [19]) to undergo metabolic fates that include the synthesis of ATP. One consequence of the activation of P1 (and certain P2Y) receptors is a change in cellular levels of cAMP, which can exit cells by certain multidrug resistant proteins (MRPs 4 and 5; [20]). The conversion of extracellular cAMP to AMP by *D. discoidium* occurs via extracellular phosphodiesterases [21]. Although similar mechanisms are not as clearly defined in mammalian tissues, metabolism of cAMP to AMP has been demonstrated in several systems (e.g., [22], [23]). In such tissues, this conversion likely occurs via ectonucleotide pyrophosphates (i.e., NPP1-3) that have phosphodiesterase activity [17].

Although extracellular ATP can promote the release of chemottractant cytokines from a variety of cells (e.g., through P2X7-mediated activation of the NALP inflammasomes; [24, 25]), ATP, and its metabolites, acting by the pathways described above, can also amplify chemoattractant signals and directly stimulate cell migration. Below, we review the modulation of chemotaxis and cell motility by nucleotides and

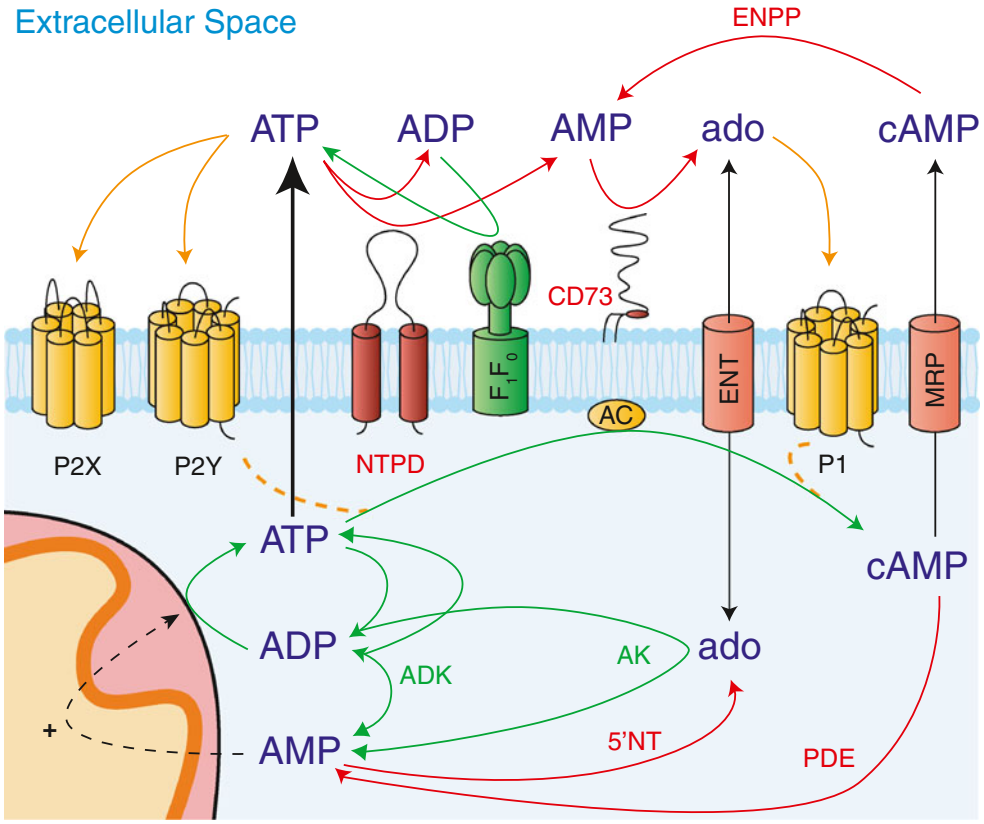

Fig. 3 P1/P2 receptor signaling pathways. A diverse array of intracellular and extracellular receptors and enzymes facilitate P1/P2 receptor signaling pathways. ATP released into the extracellular space can activate G protein-coupled P2Y receptors or P2X ion channels. Ectonucleotidases (e.g., NTPDases/CD39 and ecto-5′-nucleotidases/CD73) hydrolyze released ATP, generating extracellular ADP, AMP, and adenosine (ado). Adenosine, which can also be released from or internalized by cells through equilibrative nucleoside transporters (ENTs), can activate G-protein-coupled P1 receptors. It has been proposed that cell-surface F1F0 synthase perpetuates ATP signals by facilitating the conversion of ADP to ATP. Adenylyl cyclase, the activity of which is controlled by GPCRs, generates the second messenger cAMP, which cells can release via multidrug resistance proteins (MRPs). Extracellular nucleotide pyrophosphatase/phosphodiesterases (ENPPs) facilitate the conversion of released cAMP to adenosine. In the intracellular environment, adenosine kinase (AK), adenylate kinase (ADK), and phosphodiesterases (PDE) contribute to ATP generation, while 5′NT generates adenosine. The conversion of ADP to ATP is primarily driven by mitochondrial pathways that are upregulated as intracellular AMP concentrations are increased

adenosine and emphasize similarities and differences between professional and non-professional phagocytes. We focus on articles related to these topics that have appeared in the past 5 years (2006–2011). Table 1 provides an overview of the cell types in which P1/P2 receptors and certain effectors regulate cell migration and chemotaxis. In the subsequent text, we highlight findings for neutrophils, microglia, endothelial cells, and cancer cells.

Neutrophils

Neutrophils represent 50–75 % of circulating leukocytes [26] and are the most abundant of the body's professional phagocytes. As part of the first line of defense of the innate immune system, neutrophils detect and direct their movement in response to chemoattractant signals released by pathogens and damaged tissues. The net forward movement of neutrophils results from PI3K-mediated F-actin polymerization at the front (i.e., the leading edge or pseudopod) and the Rho GTPase RhoA-mediated contraction of actin-myosin II complexes at the back of the cell (the trailing edge, i.e., uropod) [27]. The ability of neutrophils to orient themselves in chemoattractant gradients as shallow as 1 % implies that the cells amplify external gradients [28], although the signaling pathways involved in this amplification are not fully understood.

Extracellular ATP and autocrine/paracrine P1/P2 receptor signaling pathways are critical mediators of neutrophil chemotaxis. Upon stimulation with chemoattractants, such as the bacteria-derived formyl peptide receptor agonist *f*Met-Leu-Phe (fMLP), human neutrophils release ATP in a polarized fashion at their leading edge [29]. ATP does not act as a chemoattractant but promotes chemokinesis; the chemotactic response to fMLP is inhibited if neutrophils are incubated with the ATP scavenger apyrase, indicating that this response requires extracellular ATP [29]. Apyrase also inhibits chemotaxis of mouse neutrophils in response to W-peptide (Trp-Lys-Tyr-Met-Val-d-Met; [30]), a potent agonist of the mouse formyl peptide receptors [31]. In addition, apyrase inhibits chemotaxis of macrophages promoted by the complement agonist C5a [32]. Intraperitoneal injection of apyrase attenuates neutrophil accumulation in the lungs of mice following lipopolysaccharide (LPS)-induced injury [33] and reduces the number of adherent neutrophils in the microvasculature at sites of focal hepatic injury in mice [13]. Although in vivo imaging experiments in the latter study suggested that apyrase did not affect the percentage or speed of neutrophils undergoing chemotaxis and the authors attributed the effect of apyrase to suppression of P2X7-mediated activation of the macrophage Nlrp3 inflammasome, the local concentration of apyrase may have been too low to overcome the high extracellular concentration of ATP, which can reach 80 μM [34].

Nucleotide-activated P2 receptors are key participants in the ATP-promoted chemotactic pathway of neutrophils. Exposure of neutrophils to the non-hydrolyzable ATP analog ATPγS, which overwhelms the polarized endogenous extracellular ATP signals, impedes the ability of the cells to accurately migrate in response to point sources of fMLP, thus implicating P2 receptors in the polarization of neutrophils in chemoattractant gradients [29]. Experiments with $P2Y_2$-knockout animals reveal reduced sequestration of neutrophils in the lungs in a mouse model of sepsis [29, 35]. Studies with neutrophil-like HL-60 cells suggest that $P2Y_2$ receptors may co-localize with fMLP-activated formyl-peptide receptors at the cell surface [36]. In addition, ATP and P2 signaling are required for IL-8-induced neutrophil chemotaxis in vitro [37]. P2X receptors may also contribute to the regulation of neutrophil chemotaxis: A P2X1 receptor-selective agonist can induce chemokinesis and enhance IL-8-induced chemotaxis of human neutrophils by activating RhoA, which, as noted above, promotes the contraction of actin/myosin complexes at the trailing edge of migrating cells [30].

Adenosine-activated P1 receptors modulate neutrophil chemotaxis, though their precise role is unclear. Adenosine receptors couple to G_i (A_1, A_3) or G_s (A_{2a}, A_{2b}), which can exert distinct physiological responses [38]. A_{2a} receptors inhibit neutrophil chemotaxis, as shown by studies that used selective A_{2a} agonists [39] and A_{2a}-knockout mice [40]. The role of G_i-coupled A_1 and A_3 receptors is more controversial. Early studies showed that A_1 receptors exert pro-inflammatory effects by promoting neutrophil adhesion and chemotaxis [41, 42], but more recent data obtained with knockout mice suggest that A_1 receptors inhibit neutrophil microvascular permeability [43]. Other evidence suggests that A_3 receptors localize at the leading edge of human neutrophils during chemotaxis: Inhibition of A_3 receptor signaling blunts migration in gradients of fMLP or IL-8 [29]. However, a study that used the A_3-selective agonist CP-532-903 suggested that A_3 receptor activation inhibits the migration of murine neutrophils [44]. In addition, A_3 knockout mice have reduced accumulation of neutrophils in the lungs in a model of inflammation [45]. The authors of the study that used CP-532-903 reported that this inhibition is not due to cAMP production, alterations in Ca2+ signaling, or desensitization of fMLP receptors, and speculated that activation of A_3 receptors may sequester G_i-linked signaling components that are necessary for neutrophil migration [46].

Although results of studies related to the effects of adenosine are inconsistent, together they indicate a role for A_3 receptors in the chemotaxis of neutrophils and suggest that spatiotemporal aspects of signaling contribute to this action. This idea is supported by evidence that CD39 (NTPDase1), which initiates the hydrolysis of extracellular ATP to adenosine, localizes at the leading edge of migrating human neutrophils and is

Table 1 Cell types in which P1/P2 receptors and effectors regulate cell migration and chemotaxis

Cell type	Receptor/effector	Stimulation of response (unless noted otherwise; reference number)
Astrocyte progenitor cells	$P2Y_1$	Migration [113]
Breast cancer cells	P2X7	Migration [89]
	CD73	Migration [92, 93]
	A_{2b}	Migration [93]
Cardiac fibroblasts	$P2Y_2$	Migration [114]
Corneal epithelial cells	P2X7	Migration [115]
	$P2Y_2$	Migration [116, 117]
Dendritic cells	$P2Y_2$	Chemotaxis [83]
Eosinophils chemotaxis	$P2Y_2$	VCAM-1 regulation [118] and [83, 118]; monosodium urate-stimulated Cytokine release and migration [119]
Endothelial cells	ATP	PI3K/Rho/ROCK- [77] and PI3K/Akt/mTOR-modulated [73] migration
	ADP	Rac1-modulated migration [120]
	P2	RhoA/FAK-modulated migration [75]
	$P2Y_1$	MAPK-modulated migration [71, 72]
	$P2Y_{13}$	Migration [74]
	P2X4	Migration [81]
	F1/F0 synthase	Migration [79, 81]
	MRP4	↓ Migration [121]
Glioma cells	P2X7	Migration [88]
Hematopoietic stem sells	P2Y/UTP	Migration [122]
Hepatic stellate cells	A_{2a}	↓ PDGF-stimulated chemotaxis [123]
	CD73	↓ Migration [124]
Keratinocytes	P2/ATP	HB-EGF synthesis and release, ↑ migration [125]
	$P2Y_2$	Inhibition of spreading, ↓ IGF-1/PI3K dependent migration [126, 127]
Macrophages	P2/ATP	Facilitates C5a-mediated chemotaxis [32]; ↓ 'basal' migration/LPS-induced IL-6/TNFa release [55];
	P2X7	CD44 (adhesion molecule) shedding, in P388D1 cells [128], In vivo bladder macrophage migration [129]
	A_{2a}	↓ Chemokine secretion [130], ↓ CCR7 protein expression, ↓ CCR7-stimulated migration [131]
Mesenchymal stromal cells	CD73	Migration [132]
Microglia	ATP	Chemotaxis [51–56]
	$P2Y_{12}$	PI3K-dependent [133] chemotaxis [57]
	P2X4	PI3K-dependent [133] chemotaxis [57]
	CD39	Chemotaxis [68]
	A_{2b}	↓ Chemotaxis [64]
Monocytes	$P2Y_2/P2Y_6$	Regulation of IL-8 secretion/migration [134]
Natural killer cells	$ATP/P2Y_{11}$	Inhibition of CX_3CL1- and enhancement of CXCL12-induced chemotaxis [135]
Neural progenitors	$P2Y_1$	Migration [136]
Neural stem cells	ATP/UTP	Migration [137]
Neutrophils	P2	IL-8-induced chemotaxis [37], LPS-induced chemotaxis [138]
	$P2Y_2$	Chemotaxis, orientation in chemoattractant gradients [29, 35]
	P2X1	Chemotaxis [30]
	CD39	Negative regulation of ATP-induced neutrophil trafficking [139] Chemotaxis [47]
	A_3	Chemoattractant-induced migration [29] ↓ Chemotaxis [44]
	A_1	↓ Transmigration [43]

Table 1 (continued)

Cell type	Receptor/effector	Stimulation of response (unless noted otherwise; reference number)
	A_{2a}	↓ Chemotaxis [40, 140]
NG108-15 hybridoma cells	$P2Y_2$	Migration [141]
Oligodendrocyte progenitors	$P2Y_1$	Migration [142]
Pre-adipocyte fibroblasts	P2Y	Chemokinesis/Migration [143]
Renal mesangial cells	ATP/UTP	Migration [144]
Smooth muscle cells	ATPgS	Chemotaxis [145]
	$P2Y_2$	Migration [146]
	$P2Y_{12}$	Migration of smooth muscle-like cells [147]
	CD39	Facilitation of ATP/UTP/PDGF-induced migration [148]
Spermatazoa	A_3	Motility [149]
	ATP/P2	Motility [150]

required for efficient chemotaxis in response to fMLP, IL-8, and C5a [47].

Microglia

Microglia, the resident macrophages and primary professional phagocytes of the CNS, exist in two states: a resting, surveillance state and an activated state. The conversion to the activated state depends on extracellular signals [48]. Early studies revealed that extracellular ATP promotes the formation of microglial processes that are characteristic of the surveillance state [49]. Subsequent work has shown that microglia undergo ATP- and ADP-promoted chemotaxis, which is inhibited by AR-C69931MX, an antagonist of the ADP-activated $P2Y_{12}$ receptor [50]. Additional evidence indicates that ATP/ADP promotes this chemotactic response [51–56] and that $P2Y_{12}$ receptors have a critical role in the response [57]. In addition, both $P2Y_1$ [58] and P2X4 [59] receptors have been implicated in microglial chemotactic responses. The chemotactic response of microglia to ATP appears to occur via a mechanism that is distinct from chemotaxis promoted by bradykinin [60] or C5a [53, 61]; moreover, the response to ATP may be driven by both chemotaxis and chemokinesis [53, 61]. Thus, the chemotactic response of microglia to extracellular ATP has several similarities with that of neutrophils.

ATP induces the formation of processes in resting microglia [49], but recent evidence shows that it can promote the retraction of such processes and induce chemorepulsion in activated microglia [62]. The latter response has been attributed to upregulation of A_{2a} receptors and concomitant downregulation of $P2Y_{12}$ [62] and A_3 [63] receptors. Other studies implicate an inhibitory role of A_{2B} [64] and A_3 [65] receptors in microglial migration. Microglia (akin to neutrophils) express CD39 on their cell surface, thereby enabling the clearance of extracellular ATP and facilitating the generation of adenosine [66, 67]. CD39-mediated hydrolysis of extracellular ATP is critical for this chemotaxis of microglia: Microglia isolated from CD39-knockout mice do not migrate in response to ATP or adenosine [68], but this response is restored in microglia treated with ATP and apyrase. Thus, the chemotactic response of microglia is akin to that of neutrophils and involves both P1 and P2 receptors. Altered expression of the key receptors in this process during states of microglial activity (e.g., upregulation of inhibitory receptors and downregulation of stimulatory receptors upon microglial activation) may help fine-tune potentially catastrophic inflammatory responses in the CNS. Indeed, enhancement in P2-mediated signalling and migration responses has been observed in microglia in a mouse model of status epilepticus [69].

Endothelial cells

Although endothelial cells are not professional phagocytes, their migration is an essential contributor to angiogenesis, which is important for tissue healing, development, and cancer progression [70]. Many different types of endothelial cells exhibit a chemotactic response to extracellular nucleotides; the $P2Y_1$ receptor has been implicated as a mediator of this process [71, 72]. Activation of $P2Y_1$ receptors by ADP promotes human umbilical vein endothelial cell (HUVEC) migration via mitogen-activated protein kinase pathways (including ERK1/2, JNK, and p38; [71]). Similarly, in vasa vasorum endothelial cells (VVECs), but not pulmonary artery or aortic endothelial cells, ATP promotes cell migration and potentiates the DNA synthesis-stimulating activity of VEGF and bFGF [73]. The nucleotide-induced migration of VVECs occurs via a pathway that is similar to, but distinct from, platelet-mediated pathways [74] and likely occurs by $P2Y_1$-mediated activation of ERK1/2, PI3K, and mTOR [73].

The chemotactic response of endothelial cells to extracellular nucleotides may also involve other signaling pathways.

Exposure to hypergravity induces the release of ATP and migration of bovine aortic endothelial cells in a RhoA activation/FAK phosphorylation-dependent manner [75]. Studies of HUVECs reveal that extracellular ATP and UTP both induce FAK phosphorylation and promote endothelial migration through the activation of $P2Y_2$ and $P2Y_4$ receptors [76]. In addition, extracellular ATP, via P2Y receptors, activates PI3K and Rho/ROCK pathways in VVECs, which in turn promote the release of ATP [77]. Such studies suggest that nucleotide receptor-mediated positive feedback loops may promote endothelial cell migration.

Enzymes that control the availability of extracellular nucleotides play an important role in angiogenesis. Although there has been debate regarding its contribution to extracellular ATP synthesis [78], F1F0 synthase appears to be an important positive regulator of endothelial cell migration [79–81]. Interestingly, F1F0 synthase is also the major endothelial binding site for angiostatin, a potent inhibitor of angiogenesis and endothelial cell migration [82, 83].

Cancer cells

Cancer cells can use extracellular nucleotide-mediated migration pathways to facilitate their proliferation and migration and facilitate tumor angiogenesis. Various cancer cells over-express P2X7 receptors [84–87] that regulate their motility (e.g., C6 glioma cells [88] and breast cancer cells [89]). ATP enhances the migration of TGFβ1-treated A549 lung cancer cells, although the precise receptor responsible for this effect has not yet been identified [90].

Enzymes that control the availability of extracellular ATP can play an important role in tumorigenesis. Tumor cells express F1F0 synthase on their extracellular surface; some evidence suggests that this enzyme is the target of angiostatin [91], a protein that is cytotoxic to tumors by inhibiting angiogenesis. Human breast cancer cells release an adenosine 5′-diphosphate transphosphorylase (sNDPK) that promotes the extracellular synthesis of ATP, which is proposed to induce $P2Y_1$-dependent endothelial cell migration and VEGFR-2 activation [72].

The P1 receptor pathway has also been implicated in promoting cancer cell migration; both CD73 [92, 93] and A_{2b} activity [93] can contribute to this response. Furthermore, adenosine upregulates the expression of the chemokine receptor CXCR4 in cancer cells, an effect that is proposed to occur through the activation of A_{2a}/A_{2b} receptors [94]. The extracellular pyrophosphatase autotaxin (NPP2) stimulates motility of cancer cells [95] and migration of endothelial cells [96]. Studies of autotaxin in cancer progression have focused on its lysophosphatidic acid (LPA)-producing activity [97]. However, because LPA can stimulate ATP release from human endothelial cells [98], it is tempting to speculate that, akin to what occurs in neutrophils, LPA-induced angiogenesis depends upon P2-receptor signaling.

It is not known if the elevated extracellular ATP levels in tumor microenvironments help recruit immune cells. Such recruitment contributes to the pathogenesis of cancer [99] but can also potentially be exploited to promote the destruction of cancerous tissue [100, 101]; thus, ATP signaling pathways represent potential therapeutic targets in the treatment of cancer.

Perspectives and conclusions

Many different cell types migrate in response to extracellular cues; cell migration plays critical roles in a diverse array of physiological processes, including inflammation [102], angiogenesis [103], wound healing [104], and development [105]. Cell migration can also contribute to pathology, helping to mediate autoimmune responses, developmental disorders, tumor progression, and metastasis. Thus, cell migration pathways are potential targets for the treatment of a diverse array of diseases. As noted above, autocrine/paracrine P1/P2 signaling pathways influence cell migration and as such, may be therapeutic targets for settings in which such migration is defective or excessive.

Studies of P1/P2-receptor-mediated cell migration and its possible therapeutic targeting have a unique set of challenges. The complex, multi-component nature of these signaling mechanisms makes it difficult to identify specific receptors for individual pathways, a problem that is exacerbated by the limited availability of selective P2-receptor agonists and antagonists. Certain approaches can help circumvent such difficulties. For example, the use of enzymes, such as apyrase and adenosine deaminase, to hydrolyze nucleotides and adenosine, respectively, can aid in defining their contribution to cellular events. Non-hydrolyzable analogs, such as ATPγS, can also help distinguish P1- and P2-mediated effects. Caution is required in using agents such as suramin, a P2 receptor inhibitor that also interacts with other signaling components (e.g., G-proteins [106] and protein arginine methyltransferase 1 [107]). Furthermore, because cells often express multiple P1/P2 receptor subtypes, one must employ drug concentrations that are appropriate for the receptors of interest. The use of knockout mice and siRNA/shRNA approaches are alternatives to the administration of pharmacological agents for assessing and defining the role of particular P1 and P2 receptors.

Studies of cell migration have a unique set of challenges. Transwell assays, which are often used to assess cell migration, facilitate the rapid acquisition of large amounts of data. However, the chemoattractant gradients created in transwell assays can be atypically steep [108], and the information

obtained from such assays is limited, for example, with respect to the directionality of cell migration and in differentiating chemotaxis from random movement (chemokinesis). Because of the polarized nature of signaling in cell migration, pharmacological agents can have different effects if added to the upper or lower wells of such assays, which may explain inconsistent findings (e.g., for A_3 adenosine receptors in neutrophil migration). Microscope-based 2D chemotaxis methods can yield detailed data regarding the speed and trajectory of migrating cells but are technically challenging and require specialized equipment. The latter include cell migration slides, which, although expensive, may provide useful information (e.g., [108]). Given the limitations of individual methods, investigators should seek to use multiple, complementary approaches when studying cell migration. Comprehensive overviews of currently available techniques and the advantages and disadvantages of each can be found in the reviews of Zigmond et al. [109] and Roussos et al. [99].

Considerable evidence implicates P1/P2-mediated cell migration pathways in pathophysiology [69]. For example, recent studies show that pathogens such as HIV [110] and malaria [111] promote infection by "hijacking" P1/P2 signaling pathways. These pathways may provide therapeutic targets in such settings. Interest in the possible use of inhibitors of cell migration has increased in recent years. In addition, there is an increased prevalence of autoimmune disorders, such as type I diabetes mellitus, and a growing recognition of a need for therapies that downregulate immune cell activity without abolishing the ability to remove pathogens [112]. P1/P2-mediated migration thus may offer therapeutic opportunities, in particular, if one can identify unique pathways and exploit information regarding receptor/ecto-nucleotidase stoichiometry so as to define "signaling bottlenecks" as novel therapeutic targets. Efforts directed at altering P1/P2-mediated cell migration pathways may thus prove to be therapeutically useful.

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