Review Article

Stem Cell INVESTIGATION

Expression and function of P2 receptors in hematopoietic stem and progenitor cells

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Abstract: Nucleotides have unambiguously emerged as a family of mediators of intercellular communication, which bind to a class of plasma membrane receptors, P2 receptors, to trigger intercellular signaling. P2 receptors can be further divided into P2X and P2Y subfamilies based on structure and function. Different hematopoietic cells express diverse spectrums of P2 receptors at different levels, including hematopoietic stem and progenitor cells (HSPCs). Extracellular adenosine triphosphate (ATP) exerts different effects on HSPCs, regulating cell proliferation, differentiation, migration, and chemotaxis, release of cytokines or lysosomal constituents, and generation of reactive oxygen or nitrogen species. The relationship between abnormal P2 receptor function and human diseases attracts more and more attention. This review summarizes the expression and function of P2 receptors in HSPCs and the relationship to hematopoietic diseases.

Keywords: Hematopoietic stem and progenitor cells (HSPCs); P2 receptors; nucleotides

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Introduction

The molecule adenosine triphosphate (ATP) is functional as a universal energy currency inside cells (1). However, no one envisioned a role for the molecule outside of the cell until 1962. At that time, Burnstock, as a young neurophysiologist, saw evidence of neural signaling that did not involve the classical neurotransmitter chemicals acetylcholine or noradrenalin. After more than a decade, Burnstock proposed the existence of "purinergic nerves" that released ATP as a neurotransmitter (2). Henceforth, a long series of discoveries has now demonstrated beyond doubt that ATP is a critical signaling molecule that allows cells and tissues throughout the body to communicate with one another, and that promotes a wide range of pathophysiological responses via activation of nucleotide receptors cell surface (3). Furthermore, it appears to influence a diverse scale of biological processes such as the generation of chemotactic signals, activation of different immune cells, causing inflammatory cells to migrate, proliferate, differentiate, or release diverse inflammatory mediators (4).

Purinergic receptors are divided into two families, P1 receptors and P2 receptors. In contrast to P1 receptors, which are activated by the ATP metabolite adenosine, P2 receptors are activated by ATP and/or other nucleotides (for example, UTP). Extracellular effects of nucleotides were initially recognized in smooth muscle contraction, neurotransmission, regulation of cardiac function, and platelet aggregation (5). However, over the last 10 years the intercellular mediator role of these molecules has become widespread. Hematopoietic cells express different spectrum of P2 receptors, which emerge as interesting

targets during the process of signal transduction (4). In addition to physiological process, abnormal purinergic signaling was reported to have pathophysiological roles in a variety of disease, including hematologic malignancies. In this review, we summarize the distribution of P2 receptors in hematopoietic stem and progenitor cells (HSPCs) and discuss the role of P2 signaling in HSPCs and related malignant diseases.

Classification of P2 receptors

According to the International Union of Pharmacology (IUPHAR) Committee on Receptor Nomenclature and Drug Classification, receptors for extracellular nucleotides (eNTPs) are termed P2 receptors (this nomenclature replaces the older "P_2-purinoceptor"). Based on the molecular structure other than pharmacologic and functional criteria, P2 receptors are divided into two subfamilies: ligand-gated ion channels (P2X) and G protein-coupled (P2Y) (6-8). To date, 15 mammalian P2 receptors have been cloned, characterized, and recognized as responsible for the diverse cellular responses to stimulation with eNTPs. In mammalian cells, there are seven P2X receptors denoted P2X1 through P2X7, and several spliced forms of these subunits have been identified (9). There are eight mammalian P2Y receptors denoted P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13 and P2Y14 (10). Several receptors are not included in the list of mammalian P2 receptors for different reasons. P2Y5, P2Y7, P2Y9, and P2Y10 have been purged from this sequence because they are primarily non-nucleotide receptors though they may also bind eNTPs. P2Y3 receptor, which has not been cloned from mammals but from chick brain, suggested to be a homologue of the mammalian P2Y6 (11). P2Y8 has so far only been cloned from *Xenopus* neural plate.

P2X receptors

P2X receptors are ATP-gated ion channels, originally cloned and characterized in excitable cells (12,13). P2X receptors are shown to be nearly ubiquitous that mediate fast permeability changes to monovalent and divalent cations (Na^+, K^+, and Ca^{2+}) (8,14). In mammalian cells, seven P2X (P2X1-7) receptors have been cloned and characterized pharmacologically (11). Among them P2X2 and P2X4 have two splicing subtypes. Human P2X4 and P2X7 genes are located close to the tip of the long arm of chromosome 12 (12q24.31), where 230 kb of genomic DNA also contains the gene for calmodulin-dependent kinase type II. P2X1 and P2X5 genes are also very close together (and close to the gene encoding the vanilloid receptor VR1) on the short arm of chromosome 13. The remaining genes are on different chromosomes [P2X3 genes on chromosome 11 (11q12) and P2X6 genes on chromosome 22 (22q11)] (9). P2X receptors range from 379 to 595 amino acids and have two transmembrane hydrophobic domains separated by a bulky extracellular region harbouring ten cysteines and two to six N-linked glycosylation sites (15). The amino-termini and carboxy-termini are both on the cytoplasmic side of the plasma membrane. The amino-termini is short with less than 30 amino acid residues, while the carboxy-termini varies from 25 to 240 amino acid residues. The amino acid composition among subunits of P2X (P2X1-7) receptors has a sequence homology of 26-47%.

P2Y receptors

P2Y receptors belong to the G-protein-coupled receptor (GPCR) family and contain an extracellular amino terminus, an intracellular carboxy-terminus and seven transmembrane-spanning motifs. At present, eight distinct mammalian P2Y receptors have been cloned and characterized, ranging from 328 to 379 amino acids with molecular mass of 41 to 53 kd after glycosylation (16). According to their phylogenetic and sequence divergence, two distinct P2Y receptors subgroups have been proposed. The first group includes the P2Y1, P2Y2, P2Y4, P2Y6 and P2Y11 subtypes, with a sequence homology of 35-52% in amino acid composition and the presence of a Y-Q/K-X-X-R defining motif in the transmembrane α-helix 7, which affects ligand-binding characteristics. This group is coupled to G_q/G_{11} (leading to calcium release via phospholipase C/inositol-1,4,5-triphosphate activation). By contrast, the second group contains P2Y12, P2Y13 and P2Y14 receptors, sharing a sequence homology of 47-48% and with a K-E-X-X-L motif in transmembrane α-helix 7. They inhibit activation of adenylate cyclase and modulate flow through ion channels by binding to $G_{i/o}$ proteins (16). Despite sequence homology, there are marked differences among individual members of the P2Y family regarding their intracellular signaling cascades. For example, P2Y11, a unique subtype, stimulates activation of both phosphoinositide and adenylate cyclase pathways.

Expression and function of P2 receptors in HSPCs

Purinergic signaling in hematopoiesis has mainly been investigated in terminally differentiated cells (4,17) to participate in several cell functions, including platelet aggregation (18), chemotaxis (19,20), cell death, pro-inflammatory activity (21) and so on. Despite the large number of research on purinergic signaling in immune effector cells, investigation of eNTPs-mediated responses on HSPCs started only a few years ago. Recently, more and more studies show the effects of eNTPs on HSPC proliferation, differentiation, migration, and senescence. At mRNA level, HSPCs express for all P2X receptors and some P2Y receptors including P2Y1, P2Y2, P2Y11, P2Y12, P2Y13, and P2Y14 (22).

Proliferation

eNTPs strongly stimulated proliferation of HSPCs and expanded clonogenic $CD34^+$ and Lin^- $CD34^-$ progenitors in normal physiological conditions. In 2004, Lemoli *et al.* observed that nearly all P2X and P2Y receptors were expressed on $CD34^+$ hematopoietic progenitors (23). Hematopoietic stem cells (HSCs) were isolated from three sources: steady-state BM, cord blood, and mobilized peripheral blood (PB). *In vitro* stimulation of HSCs with low concentration of ATP and, to a higher extent, UTP, induced fast release of intracellular calcium, and mediated fast changes in the intracellular ion homeostasis. Furthermore, eNTPs also enhanced the stimulatory activity of several cytokines on clonogenic $CD34^+$ and Lin^- $CD34^-$ progenitors and expanded more primitive $CD34^+$-derived long-term culture-initiating cells (LTC-ICs). Interestingly, *in vivo* experiment also demonstrated that engraftment of $CD34^+$ HSCs, which short-termly incubated with UTP, to sublethally irradiated NOD/SCID mice extremely expanded the number of human BM-repopulating $CD34^+$ cells (23). In 2011, similarly results were obtained by Casati *et al*. showing that ATP positively affected cell-cycle dynamics in $Lineage^-$ c-Kit^+ $Sca\text{-}1^+$ (LKS^+) HSPCs in a cell-autonomous manner, and ATP actively accumulated within cytoplasmic vesicles in murine HSPCs (24). On stimulation of Ca^{2+}-sensitive pathways, HSPCs can release these vesicles, igniting a positive autocrine loop, which involves P2X1 and P2X4 subtypes, further lead to ATP release and HSPCs proliferation. The role of endogenous ATP on proliferation of HSCs and LKS^+ becomes more prominent under inflammatory conditions. As assessed in two mouse models of T-cell-mediated chronic inflammation, namely calreticulin (crt)-deficient fetal liver chimera (FLC) and mice with inflammatory bowel disease (IBD), activation of P2X receptors was significantly restrained after treatment with P2X antagonist periodate-oxidized 2',3'-dialdehyde ATP (oATP) for 5 days (24). Notably, proliferation of HSCs and LKS^+ cells were significantly reduced by oATP treatment. Cell cycle analysis revealed the substantial decrease of cells in S/G2/M phases upon treatment with oATP. All these studies indicate that P2X activation crucially contributes to HSCs expansion during inflammation, thus pointing to a function for extracellular ATP as a regulator of HSCs population size.

Differentiation

Investigation of purinergic signaling in hematopoiesis refers to lineage differentiation in early studies. Subsequently, new evidences emerged supporting the widespread expression of P2 receptors in hematopoietic cells. The differentiation of myeloid, erythroid, megakaryocytic, and lymphoid progenitors has been shown to be influenced by eNTPs, although different P2 receptors appear to be involved, depending on the specific lineage (25).

Early studies have shown the role of extracellular ATP in the differentiation of HL-60 and NB4 human promyelocytic leukemia cells into neutrophil-like cells during myeloid differentiation (26-28). It was demonstrated that ATP and several synthetic analogues induced the formation of mature, neutrophil-like cells through the stimulation of cAMP accumulation and with a ligand rank order of potency characteristic of the P2Y11 receptor (29). In addition, they also showed that ATP-induced increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) promoted transient proliferation and induced the differentiation of primitive hematopoietic cells in long-term bone marrow cultures (LTBMCs) (30). Afterwards, Barbosa *et al*. further reported that administration of ATP *in vivo* had a tuning role on myeloid differentiation, especially on more immature myeloid progenitors (31). When freshly isolated HSCs and myeloid precursor cells (CMP, GMP and MEP) from bone marrow were stimulated with ATP, the percentages of HSC, CMP and GMP populations were reduced, whereas the MEP population remained unchanged. Subsequently, *in vivo* experiments showed that treatment with ATP for 4 days led to a reduction in the number of myeloid precursor

cells and a corresponding increase in the mature myeloid population (Gr1$^+$, Mac1$^+$) (31). In addition, the presence of ATP in hematopoietic niches is an important factor in regulating HSC activity in combination with hematopoiesis regulating cytokines, such as IL-3, SCF, GM-CSF, which can modulate ATP-induced differentiation.

Apart from the above-mentioned P2Y receptors, Gpr171 is an orphan GPCR which has been identified as P2Y-like receptor for eNTPs. Rossi *et al*. reconstructed Gpr171 phylogenesis in mice and confirmed that Gpr171 was evolutionally related to members of a P2Y gene-cluster localized on mouse chromosome 3 (32). As opposed to other P2Y receptors, they found that Gpr171 expression was down-regulated in monocytes and granulocytes, suggesting a negative role in myeloid lineage specification. When Gpr171 was over-expressed in a myeloblastic cell line (32D cells) and in primary Sca-1$^+$ hematopoietic progenitors, decreased expression of myeloid markers (Mac-1, F4/80, CD16/32 and CD14) and increased formation of colonies was observed upon activation of Gpr171 *in vitro*. Conversely, Gpr171 silencing induced opposite results, i.e., diminishing the expression of myeloid progenitor markers and the clonogenic potential of 32D cells. *In vivo*, mice transplanted with hematopoietic progenitor cells over-expressing Gpr171 displayed a significant reduction in the percentage of Mac-1$^+$ Gr-1$^+$ cells. All these findings indicate that the P2Y-like receptor Gpr171 negatively regulates myeloid differentiation.

Migration

Under physiological conditions, HSCs are believed to primarily dwell within the nurturing environment of the stem cell niche. The niche shields HSCs from external injury and helps maintain their survival, quiescence, and self-renewal. Nevertheless, HSCs have been found to circulate in the PB and other tissues as well. It was suggested that circulating HSCs are actually patrolling peripheral tissues, looking for potential injuries and infections (33). Nonetheless, migrating HSCs have an advantage over BM-resident HSCs: they may act as scouts in the peripheral tissues and sense the presence of pathogens directly at infection sites, possibly promoting a rapid and localized production of immune cells (34). Recently, eNTP have been shown to modulate HSC migration in the presence of CXC-chemokine12 (CXCL12), an important chemotactic factor for HSCs and responsible for stem cell retention in the BM niche. Extracellular UTP (eUTP) improved migration of human HSCs toward CXCL12 gradients and inhibited reduction of CXCR4 in migrating CD34$^+$ cells (35). Similarly, pretreatment with eUTP significantly increased BM homing of CD34$^+$ HSCs when transplanted into immunodeficient mice. Of note, purinergic signaling also indirectly affects migration of HSPCs, by acting on the HSC niche. BM-derived mesenchymal stromal cells (BM-MSCs) represent a key component of the hematopoietic niche and secrete several HSC regulatory molecules, such as CXCL12 and stem cell factor. Recent findings showed that ATP treatment was associated with an increase in the production of pro-inflammatory cytokines in BM-MSCs, and the expression of purinergic receptors was modulated during adipogenic and osteogenic differentiation (36,37).

Senescence

P2Y-dependent signaling contributes to thrombotic and/or inflammatory consequences of tissue injury by altering platelet and endothelial activation and immune cell phagocytosis. Recently, Cho *et al.* demonstrated that P2Y14 mediated anti-senescence effects through $G_{i/o}$ protein-dependent pathway, by monitoring and responding to the extracellular manifestation of tissue stress, thus prevented the premature decline of regenerative capacity after injury (38). Under physiological conditions, P2Y14 appears to be neither essential for normal embryonic development nor for the maintenance of tissue homeostasis in the adult organism. However, the embryo is exceptionally sensitive to radiation-induced damage, and under the stress conditions of total body irradiation (TBI), P2Y14 KO embryos were more prone to undergoing IR-induced senescence than WT embryos. In the adult, hematopoietic cells are among the most sensitive cells to radiation injury. P2Y14 KO mice were more sensitive to radiation, showing a more severe reduction in the number of BM cells than that observed in WT mice. This was accompanied by a more severe reduction of LSK cells in P2Y14 KO mice, suggesting that loss of P2Y14 conferred increased susceptibility to radiation in HSPC populations. Together, radiation stress, aging, sequential exposure to chemotherapy, and serial bone marrow transplantation increased senescence in animals lacking P2Y14. Enhanced senescence coincided with increased ROS, elevated $p16^{INK4a}$ expression, and hypo-phosphorylated Rb and was inhibited by treatment with a ROS scavenger or inhibition of p38/MAPK and JNK (38).

Expression and function of P2 receptors in malignant hematopoiesis

There is growing interest in abnormal expression and dysfunction of P2 receptors in tumor cells. Different P2 receptor subtypes are involved in the growth inhibitory response observed in the different malignant cell types challenged with ATP or other nucleotides (39). However, it is likely that the final effect is caused by the combination of contributions from multiple P2 receptor subtypes than a single subtype. For example, two different P2Y receptor subtypes were proposed to be responsible for the increase in $[Ca^{2+}]_i$ in HL-60 cells, P2Y2 (or P2Y4) receptor and probably P2Y1 receptor (40). ADP and ATP increased $[Ca^{2+}]_i$ in CB1 cells which isolated from a patient with T-ALL, probably via P2Y1, P2Y12 or P2Y13 receptors (41). To date, five P2 receptor subtypes have primarily been implicated in the growth inhibition of tumor cells, namely P2X5, P2X7, P2Y1, P2Y2 and P2Y11 (42), however, the significance of these receptors differ among cell lines. Among P2 receptors, the P2X7 subtype is most widely accepted as the purinergic receptor mediator of apoptotic or necrotic cell death as initially suggested by early experiments in mouse tumor cell lines where ATP was shown to trigger cell death via a necrosis or apoptosis. However, analysis of the effect of the P2X7 receptor on tumor growth made it more complex by the observation that tonic, as opposed to pharmacological, stimulation may have a trophic, growth-promoting, rather than cytotoxic effect in leukemia cells (43), such as chronic lymphocytic leukemia (CLL) (44,45), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML).

In pediatric AML patients, Chong *et al.* reported that P2X1, P2X4, P2X5 and P2X7 receptors were up-regulated compared with normal controls and the highest expression of P2X7 receptors was found in relapsed patients, while P2X2, P2X3 and P2X6 receptors were absent or marginally expressed (46). A significant decrease in the expression of P2X4, P2X5 and P2X7 receptors was observed after complete remission after chemotherapy (46). Salvestrini *et al*. analyzed the expression levels of 15 cloned human P2X and P2Y receptors in U937 and OCI-AML3 cell lines and in primary blasts from 15 leukemia patients (47). The results showed that OCI-AML3 and U937 cells expressed the P2X1, P2X4, and P2X7 subtypes. U937 cells expressed all P2Y subtypes, whereas P2Y1 and P2Y12 were not detected in OCI-AML3 cells. Primary leukemic cells expressed P2X1, P2X4, P2X5, and P2X7, whereas P2X2, P2X3, and P2X6 were not detected. The P2X7R mRNA level was significantly higher in AML cells than in normal $CD34^+$ cells. The abnormal expression of purinergic receptors in leukemia cells suggested a wide dysregulation and a possible pathogenic role of purinergic signaling in hematologic malignancies.

Besides over-expression of P2X7 in leukemia and tumor cells, evidence from *in vitro* and *in vivo* studies indicated that the addition of exogenous ATP to leukemia and colon cancer cells inhibited cell growth (39). Hence, the pro-apoptotic effects of P2X7 receptor was firstly proposed in early studies. The binding of ATP induced within milliseconds the opening of a channel selective for small cations, and then within seconds a larger pore non-selectively permeable to molecules with a mass of up to 900 Da, resulting in cell apoptosis (48). Then, Salvestrini *et al.* showed that activation of P2X7 influences cell cycle. Extracellular ATP binding to abnormal over-expression P2X7 in leukemia cells, may drive a negative feedback loop regulating the expression of P2X7 itself, thus counteracting cell growth by the preferential expression of growth-arrest genes and cell-cycle inhibitors (such as CDKN1A/p21, waf1 and G0S2) and down-regulating the expression cell-cycle related genes (such as cyclins and CDKs) and transcription factors involved in cell proliferation (MYB, MYC and GFI1). This changes on expression of cell-cycle related genes resulted in a cell-cycle arrest in the G0 phase, and a decrease of G1- and S-phase cells, and finally inhibition of AML proliferation and CFU-Ls formation (47). Moreover, a series of P2X7 polymorphisms have been discovered, and their impacts on P2X7 functions, mechanisms, and relationship with diseases were studied in a number of variants (49). Among several loss-of-function polymorphisms (50-52), a hyposensitive mutant is also found in leukemia cells (53), whose DNA sequencing analysis revealed a substitution of A559 with G, causing an N187D substitution. This mutation is hyposensitive to its ligand, and leukemia cells bearing this P2X7 mutant have a greater growth potential *in vitro* experiment and in a nude mouse model. Furthermore, elevated angiogenesis and CD206-positive macrophage infiltration could be detected in tumor tissues formed by K562 cells bearing this mutant (53).

Extracellular ATP affect the motility of cells derived from AML patients (47). In migration assays, ATP inhibited the spontaneous migration of AML cells *in vitro* whereas UTP also reduced CXCL1-driven migration. P2Y2 and P2Y4 receptors appeared to be the primary subtypes involved

in this process. ATP treatment induced the expression of genes involved in the negative regulation of cell motility (such as TIMP1) and inhibitors of GTPase activity (such as CDC42EP2, RGS1, and RGS2). Consistently, ATP decreased many activators of cell motility (such as Rho-GTPase regulators and molecules involved in cellular migration), Interestingly, AML BM homing and long-term engraftments are also decreased upon exposure to eNTPs. Remarkably, ATP and UTP, as well as P2Y-agonists, significantly inhibited the long-term engraftment of highly purified $CD38^{-}CD34^{+}$ leukemic stem cells, suggesting that purinergic signaling can also affect rare leukemic stem cells, which usually escape most therapeutic approaches, making AML difficult to eradicate.

In addition to abnormal expression and dysfunction of P2 receptors in tumors, cells within tumor microenvironment also have altered expression of P2 receptors and exhibited the surprising function. Recently, there are some new reports about the amusing role of P2X7 in tumorgenesis. Adinolfi *et al.* investigated the effect of host genetic deletion of P2X7 in the mouse on the growth of B16 melanoma or CT26 colon carcinoma cells. They showed that host P2X7 expression was critical to support an antitumor immune response, and to restrict tumor growth and metastatic diffusion (54). Hofman *et al.* revealed an unexpected role for P2X7 in preventing colitis-associated cancer (CAC) (55). The proliferative effects of P2X7 blockade were associated with an increased production of TGFβ1 that was sufficient to stimulate the proliferation of intestinal epithelial cells. Finally, P2X7 blockade altered immune cell infiltration and promoted Treg accumulation within lesions of the digestive system. Hence, based on the apparently contradictory evidence, the precise role of P2X7 *in vivo* in the context of inflammation-associated carcinogenesis needs to be carefully addressed, and the use of P2X7 inhibitors to treat IBD give the possibility of increasing risks CAC as a result.

Similar to the above-mentioned, not only abnormal expression and dysfunction of P2 receptors were found in leukemia cells, cells within leukemia microenvironment also have altered expression of P2 receptors. In Notch1-induced leukemia model, during the development of leukemia, the expression levels of multiple subtypes of P2X in macrophages from bone marrow, spleen and peritoneal cavity were changed. In particular, P2X7 expression in macrophages from these microenvironments significantly increased. Abnormal expression and function of P2X7 could be associated with apoptosis of macrophages in late stage of leukemia (56). Abnormal Expression of P2X receptors on macrophages family during leukemia development needs to be further clarified.

There are several signaling pathways and second messenger systems being coupled to P2 receptor signaling in malignant hematopoiesis. The activation of P2X receptors resulted influx of extracellular calcium and elicit intracellular signaling pathways. The abnormal activity of P2X7 receptor was described. For instance, an N187D point mutation was detected in P2X7 receptor from a leukemia cell line resulting hyposensitive to stimulation (53). The most common signaling pathway described for P2Y receptors is an increase in PLC activity, resulting in inositol 1,4,5-trisphosphate (IP3) mobilization and the release of Ca^{2+}. Subsequently, the increase in the intracellular level of Ca^{2+} triggers changes in cell proliferation (27,44,57). In addition, P2Y receptors couple to adenylyl cyclase and might cause a change in intracellular cAMP levels. However, it is not clear which P2Y receptors activate or inhibit the production of cAMP and opposite effects of some receptor subtypes have been described in different cancer cell lines. The activation of the apoptotic caspase enzyme cascade by P2 receptor is also reported in cancer cell lines (58-61).

Future perspectives

Receptors for eNTPs are widely expressed in hematopoietic cells. They mediate a large array of signal pathways participating in a variety of physiological and pathological processes, ranging from growth stimulation to apoptosis, from chemotaxis to cell differentiation and from nociception to cytokine release, as well as neurotransmission. Therefore, further clarification of mechanisms of P2 receptors in blood diseases, their roles in clonal evolution and co-evolution in hematological malignancies will provide new insight for therapy of hematological malignancies. In fact, some clinical trials, targeting P2 receptor common agonist ATP, have been proposed. Among them, some trials involved patients with advanced cancer (62-64). Recently, a new avenue of investigation targeting P2 receptors with specific agents rather than ATP has opened up. Several examples have reported using this new strategy though no report has been focused in hematopoietic malignancies to date. Clopidogrel, a P2Y12 antagonist that inhibits platelet aggregation, is used for thrombosis and stroke (65). Diquafosol, a P2Y2 agonist, is used to treat dry eye (65). In the future, either targeting ATP or using P2 receptor subtype-specific compounds would be promising ways in the treatment of cancer and hematopoietic malignancies with up-regulated

level of signaling by P2 receptors.

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Footnote

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