P2 receptors and cancer

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Purinergic signalling has been implicated in many biological processes, and ATP and other extracellular nucleotides might have therapeutic potential in the treatment of cancer by signalling through P2 receptors. Different P2 receptor subtypes have been identified in a variety of different cancer types, in both primary samples of human cancer tissue and cell lines. Recent evidence suggests that different receptor subtypes mediate different pathophysiological functions such as proliferation, differentiation and apoptosis. *In vivo* studies of the use of ATP suggest that it can decrease the rate of cancer growth, and the first clinical trials have been undertaken. Thus, agents acting at P2 receptors might provide novel therapeutic tools in the treatment of cancer. In this article, background information about purinergic signalling and purinoceptor subtypes will be provided and then the proposed role of ATP in different cancers will be discussed in detail, including a discussion of *in vivo* studies and animal models, clinical trials and the specific P2 receptor subtypes involved.

Purinergic signalling

The concept that ATP is an extracellular signalling molecule, in addition to having an intracellular role in cell metabolism and as an energy source, took a long time to be accepted [1]. With the development of new research tools, such as molecular cloning, purinergic signalling has become an established principle not only in the rapid signalling involved in neurotransmission, but also in a wide range of other tissues and biological processes, including cell proliferation, differentiation and apoptosis in tissues as diverse as the skin, skeletal muscle, bone and the immune system [2]. In the 30 years since purinergic signalling was first proposed, the concept of the purinoceptor has been transformed from an idea into a therapeutic target in clinical practice [3], and receptor subtypes for extracellular nucleotides have been cloned in mammalian species. Purinoceptors were originally classified on the basis of pharmacology and function and were first divided into P1 receptors, with adenosine as the main ligand, and P2 receptors, with ATP and ADP as the main ligands. P2 receptors were later subdivided into ionotropic P2X and metabotropic P2Y subtypes [4]. Currently, seven P2X and eight P2Y receptor subtypes have been cloned and characterized.

P2X receptors are formed by three P2X receptor subunits. Each subunit consists of two transmembrane domains, separated by an extensive N-glycosylated extracellular loop that always contains ten cysteine residues, and intracellular amino (N) and carboxy (C) termini. These subunits can form either homomeric (consisting of only one type of receptor subunit) or heteromeric (consisting of more than one type of subunit) receptors. P2X receptors are nonselective cation channels. Under normal physiological conditions, P2X receptor activation will result in Na^+ and Ca^{2+} influx and K^+ efflux across the cell membrane, which leads to depolarization of the plasma membrane and an increase in the concentration of intracellular Na^+ and Ca^{2+}. Membrane depolarization can in turn activate voltage-gated channels, causing firing of action potentials.

To date eight P2Y receptor subtypes have been cloned in human cells: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$. These receptors are highly diverse in their amino acid sequences and their profiles for endogenous ligands [5]. P2Y receptors contain seven transmembrane domains, with an extracellular N-terminus and an intracellular C-terminus. Several secondary messenger transduction pathways have been implicated in P2Y receptor signalling. These include activation of phospholipase C (PLC), inhibition and stimulation of adenylyl cyclase and direct modulation of ion channel function.

Both P2X and P2Y receptors are expressed widely in the body, and their pharmacological properties and tissue distribution have been described [2]. *In situ* hybridization, immunohistochemistry and functional studies have demonstrated that P2X receptors are expressed not only in excitable cells such as neurons and smooth muscle, but also in non-excitable cells such as epithelial cells of the skin, lungs, gut, kidneys, vascular smooth muscle and endothelium.

Much is now known about the ectonucleotidases that break down ATP released from non-neural cells and neurons [6,7]. Several enzyme families are involved, including: ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases), of which NTPDase1, 2, 3 and 8 are extracellular; ectonucleotide pyrophosphatase (E-NPP) of three subtypes; alkaline phosphatases; ecto-5'-nucleotidase; and ectonucleoside diphosphokinase (E-NDPK). NTPDase1 hydrolyses ATP directly to AMP and UTP to UDP, whereas NTPDase2 hydrolyses ATP to ADP and 5'-nucleotidase hydrolyses AMP to adenosine.

ATP and cancer

In vivo *studies using animal models*

The anticancer activity of adenine nucleotides was first described in 1983 [8]. In these *in vivo* studies, mice inoculated with cancer cells were given systemic ATP. The

results clearly showed that daily intraperitoneal injections of ATP significantly inhibited tumour growth, prolonged survival time and inhibited weight loss (cachexia). In the original *in vivo* studies of the effects of ATP on cancer in mice, daily intraperitoneal injection of 1–2 mg ATP per gram body-weight resulted in a circulating plasma level of 3–5 μM ATP 1–5 h post-injection, which significantly inhibited tumour growth in animals with CT26 colon tumours [9–11]. In addition, a synergistic action of ATP when administered with established chemotherapeutic agents or radiotherapy has been demonstrated [12,13]. However, the mechanism through which ATP acts in these animal models has yet to be resolved. The working hypothesis of this review is that the action of ATP on extracellular P2 receptors causes an anticancer effect (discussed in detail in this article). However, two other possible mechanisms of action need to be mentioned. ATP is degraded to adenosine by ectonucleotidases, and thus increased levels of adenosine might also activate P1 receptors and result in anticancer effects [14]. Furthermore, administration of ATP (and also AMP) *in vivo* causes an expansion in the ATP pool in both erythrocytes [15] and hepatocytes [10]. Therefore, the anticancer effects, particularly the prevention of cachexia, might be modulated by this increase in intracellular (nutritional) ATP action rather than an extracellular (pharmacological) action of ATP on P2 receptors.

In vitro *studies of different cancer types*

In vitro studies of purinergic signalling in cancer have sought to determine which P2 receptor subtypes might be present in cancer cells and tissues, and the mechanism by which these receptors mediate their effects. Purinergic signalling has been studied in many different types of cancer [16], and ATP and its analogues have been shown to alter the growth of both primary cultures and human cell lines of various cancer types. A range of cellular and molecular techniques have been used to demonstrate the expression of P2 receptor protein (immunocytochemistry and western blots), mRNA [reverse transcriptase polymerase chain reaction (RT–PCR) and northern blots] and functional responses to ATP (intracellular Ca^{2+} levels and apoptotic enzyme assays). The most investigated types of cancer are discussed individually below and the results of studies of these and other cancer types are summarised in Table 1.

The expression of P2 receptors in melanoma has been studied in both paraffin-embedded specimens of excised melanomas and the A375 melanoma cell line [17,18]. $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptor mRNA has been detected using RT–PCR; however, no protein for the $P2Y_4$ receptor has been identified using polyclonal antibodies. Agonist and antagonist studies suggest that the $P2Y_1$ receptor subtype mediates a decrease in cell number whereas the $P2Y_2$ receptor subtype mediates an increase in cell number. There is no alteration in cell proliferation mediated through activation of $P2Y_6$ receptors. The alterations in cell number are most likely to be mediated through a PLC-coupled increase in intracellular Ca^{2+} levels. In addition, $P2X_7$ receptor mRNA and protein have been identified; activation of this receptor, by specific agonists, causes cell apoptosis, which has been demonstrated using YO-PRO-1 dye and caspase enzyme assays.

Non-melanoma skin cancers of keratinocyte origin express functional $P2Y_1$, $P2Y_2$, $P2X_5$ and $P2X_7$ receptors [19]. Colocalization of $P2Y_2$, $P2X_5$ and $P2X_7$ receptors with markers of proliferation, differentiation and apoptosis, respectively, has been demonstrated in excised specimens of cutaneous squamous cell carcinomas and the A431 cell line. In addition, application of receptor-specific agonists causes an increase in cell number via $P2Y_2$ receptors and a decrease in cell number via $P2X_7$ receptors.

Different research groups have described different actions of P2 receptors in colorectal cancer [20,21]. In primary cultures from seven patients and the HT 29 cell line, functional $P2Y_2$ receptors were implicated in the inhibition of cell proliferation [21]. In a different study [20], using the HCT8 and Caco-2 cell lines, which investigated the expression of all the currently established P2 receptors, activation of $P2Y_2$ receptors stimulated cell proliferation, with $P2Y_1$ and $P2X_7$ receptors mediating a decrease in cell number. These differences are discussed below.

Squamous cell carcinoma of the oesophagus has been investigated using primary tissue obtained at biopsy in conjunction with the Kyse-140 oesophageal cancer cell line [22]. $P2X_4$ and $P2Y_2$ receptor mRNA was found in both the tissue samples and the cell line but only $P2Y_2$ receptor protein was expressed. There was a functional response to $P2Y_2$ receptor activation, which resulted in an increase in the intracellular level of Ca^{2+} and a decrease in cell number over time.

In a single lung cancer cell line, A549, the expression of functional $P2Y_2$ and $P2Y_6$ receptors has been established [23]. mRNA for both these receptor subtypes was shown to be present but no analysis of protein expression was performed. The presence of functional receptors was based on an increase in cell proliferation in response to treatment with receptor-specific agonists.

Brain tumours such as gliomas and astrocytomas express functional $P2Y_1$ and $P2Y_{12}$ receptors in several different cell lines [24–26]. These receptors have been linked to a variety of different second messenger systems, including inhibitory G proteins and activation of caspase enzymes. Whereas the $P2Y_{12}$ receptor appears to increase cell number, the $P2Y_1$ receptor has opposite effects in different cell lines, causing either an increase or a decrease in the cell population.

Functional $P2X_7$ receptors have been studied intensively in primary cultures of human cervical endothelial cells and the CaSki cervical cancer cell line [27]. Treatment of these cells with ATP and 2′- and 3′-O-(4-benzoyl-benzoyl)ATP (BzATP), a more specific $P2X_7$ receptor agonist, led to an increase in cell apoptosis with increased expression of the apoptosis-linked proteins caspase-3 and caspase-9 enzymes and increased terminal deoxynucleotide transferase-mediated deoxyuridine triphosphate nick end-labelling (TUNEL).

ATP was originally shown to cause a decrease in breast tumour cell growth in MCF-7 cells at high doses (100 μM to 1 mM), which was mediated by a rise in intracellular levels of Ca^{2+} [28,29]. More recently, activation of $P2Y_2$

Table 1. P2 receptor subtype expression in different cancers[a]

Cancer type	Primary tissue	Cell line	mRNA	Protein	Second messenger system or functional response	Change in cell number	Refs
Melanoma	Yes	A375	$P2X_7$ $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$	$P2X_7$ $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$	Caspase-3,-7 PLC-mediated change in $[Ca^{2+}]_i$	↑ $P2Y_2$ ↓ $P2Y_1$, $P2X_7$	[17,18]
Skin (squamous cell carcinoma)	Yes	A431	Not investigated	$P2X_5$, $P2X_7$ $P2Y_1$, $P2Y_2$	Caspase-3, TUNEL PCNA	↑ $P2Y_2$ ↓ $P2Y_1$, $P2X_5$, $P2X_7$	[19]
Colorectal	Yes	HT29 HCT8 CaCo-2	$P2X_1$, $P2X_4$, $P2X_5$, $P2X_7$ $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$	$P2X_1$, $P2X_4$, $P2X_7$ $P2Y_1$, $P2Y_2$	$[Ca^{2+}]_i$ Cell death, ELISA	↑ or ↓ $P2Y_2$ ↓ $P2Y_1$, $P2X_7$	[20,21]
Oesphageal	Yes	Kyse-140	$P2X_4$, $P2X_5$ $P2Y_2$	$P2Y_2$	Caspase-3 PLC-mediated change in $[Ca^{2+}]_i$, TUNEL	↓ $P2Y_2$	[22]
Lung	No	A459	$P2Y_2$, $P2Y_6$	Not investigated	CaMKII, NF-κB, $[Ca^{2+}]_i$	↑ $P2Y_2$, $P2Y_6$	[23]
Prostate	No	PC-3 DU145	$P2X_4$, $P2X_5$, $P2X_7$ $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{11}$	$P2X_4$, $P2X_5$, $P2X_7$ $P2Y_2$	PLC-mediated change in $[Ca^{2+}]_i$, TUNEL	? $P2Y_2$ ↓ $P2X_5$, $P2X_7$	[29,43,49]
Brain tumours	No	1321N1 C6 U-251MG U138-MG U-87MG	$P2Y_1$, $P2Y_{12}$	$P2Y_1$	Caspase-3 ERK PLC-mediated change in $[Ca^{2+}]_i$ Adenylyl cyclase, G_i	↑ or ↓ $P2Y_1$ ↑ $P2Y_{12}$	[24–26]
Cervical	Yes	CaSki	Not investigated	$P2X_4$, $P2X_7$ $P2Y_2$	Caspase-9, TUNEL	↓ $P2X_7$	[27]
Breast	No	MCF-7 Hs578T SK-Br3 T47-D MDA-MB-231	$P2Y_2$ $P2X_7$	$P2X_7$	$[Ca^{2+}]_i$ $[K^+]_i$	↑ $P2Y_2$	[28–30,50]
Ovarian	No	OVCAR-3 EFO-21 EFO-27	$P2Y_2$	Not investigated	$[Ca^{2+}]_i$	↑ or ↓ $P2Y_2$	[30,31]
Endometrial	No	HEC-1A Ishikawa	$P2Y_2$	Not investigated	PLC-mediated change in $[Ca^{2+}]_i$,	↑ $P2Y_2$	[51]
Haematological malignancies	Yes	HL-60 NB-4	$P2X_7$ $P2Y_{11}$	$P2X_7$	$[Ca^{2+}]_i$ Adenylyl cyclase PKA	↓ $P2X_7$	[34–36]
Bladder (TCC)	No	HT-1376	Not investigated	Not investigated	Not investigated	↓ $P2Y_{11}$, $P2X_4$, $P2X_5$	[52]
Thyroid	No	ARO	$P2Y_1$, $P2Y_2$	Not investigated	PLC-mediated change in $[Ca^{2+}]_i$,	↑ $P2Y_1$, $P2Y_2$	[53]

[a]Abbreviations: CaMKII, calmodulin-dependent protein kinase II; ERK, extracellular signal-regulated kinase; NF-κB, nuclear factor κB; PCNA, proliferating cell nuclear antigen; PKA, protein kinase A; PLC, phospholipase C; TUNEL, terminal deoxynucleotide transferase-mediated deoxyuridine triphosphate nick end-labelling.

receptors in the same cell line has been suggested to cause an increase in cell number. This discrepancy is probably due to different P2 receptors being present, and these receptors becoming functional at different concentrations of ATP. In addition, there is increased expression of $P2X_7$ receptors in breast tissue undergoing malignant change [30].

There is a similar discrepancy in the study of the effects of ATP on ovarian cancer cell lines. One group has suggested that ATP causes an increase in the rate of OVCAR-3 cell proliferation mediated by the entry of extracellular Ca^{2+} into the cell [31]. An alternative report implicates $P2Y_2$ receptor activation that leads to an attenuation of cell proliferation [32].

A wide variety of P2 receptors have been reported in blood cells [33] and the immune system [34]. Functional $P2X_7$ and $P2Y_{11}$ receptors have been demonstrated in lymphocytes from patients with chronic lymphocytic leukaemia (CLL) and the HL-60 CLL cell line [35–37]. It has been suggested that the $P2X_7$ receptor causes cell death by increasing intracellular levels of Ca^{2+} and the $P2Y_{11}$ receptor is coupled to adenylyl cyclase and mediates cell differentiation.

Clinical trials

The study of purinergic signalling in cancer and whether ATP or its analogues can be used as a therapeutic agent has led to clinical trials. Three such trials have been reported to date; these trials involved the treatment of small groups of patients with advanced cancer, mainly inoperable non-small-cell lung cancer [38–40].

The first was a Phase I trial of intravenous administration of ATP to 14 patients with advanced, inoperable non-small-cell lung cancer [39]. This trial established that prolonged infusions of ATP are feasible with acceptable toxicity and that 50 μg kg^{-1} per minute is both the maximally tolerated dose and the most appropriate dose rate for subsequent Phase II testing of 96-h infusions of ATP in patients with advanced cancer. Side-effects were however observed at high doses and were related to cardiovascular disturbances such as headaches, flushing, chest tightness and dyspnea (shortness of breath, difficult or laboured breathing).

The second human clinical trial was a Phase II multicentre study of 15 patients with non-small-cell lung cancer [40]. This used the treatment regime postulated above [39]. However, in this trial, the authors found that

ATP at this dose and schedule of administration had no effect in this study.

The third trial [38] was a randomised Phase III trial of 52 patients with non-small-cell lung cancer who had not responded to previous chemotherapy or radiotherapy. They were divided into two groups: a control group and an ATP-treated group. This study showed that ATP as a single therapy did not lead to cancer regression but it did prolong survival in a subset of non-small-cell lung cancer patients at stage IIIB (characterized by high weight loss) by reducing weight loss. This subgroup, however, consisted of a small number of patients and the results therefore require confirmation in a larger trial.

The published clinical trials to date have established that systemic administration of ATP is a safe agent that might have potential as a useful anticancer agent.

P2 receptor subtypes and cancer

Primarily, five P2 receptor subtypes, the metabotropic $P2Y_1$, $P2Y_2$ and $P2Y_{11}$ receptors and the ionotropic $P2X_5$ and $P2X_7$ receptors, have been implicated in cancer to date. Alteration of cancer cell number might be due to modulation of cell proliferation via $P2Y_1$ and $P2Y_2$ receptors, stimulation of differentiation with subsequent inhibition of proliferation via $P2X_5$ and $P2Y_{11}$ receptors or induction of cell death (apoptosis) via $P2X_7$ receptors.

P2Y₁ and P2Y₂ receptors

$P2Y_1$ and $P2Y_2$ receptors are, perhaps, the most widely studied purinoceptor subtypes in cancer. Their pharmacological characterization is possible with the use of relatively specific agonists 2-methylthioADP (2-MeSADP) for $P2Y_1$ receptors and uridine $5'$-triphosphate (UTP) for $P2Y_2$ receptors, and antagonists such as MRS2179 (see Chemical names) for $P2Y_1$ receptors [4]. The main signal transduction pathway of $P2Y_1$ and $P2Y_2$ receptors is PLC activation, which leads to the formation of inositol (1,4,5)-trisphosphate $[Ins(1,4,5)P_3]$ and the mobilization of Ca^{2+} from intracellular stores such as the endoplasmic reticulum. A second signalling pathway for these receptors might be the inhibition of adenylyl cyclase, and other pathways such as calmodulin-dependent protein kinase II (CaMKII) and nuclear factor-κB (NF-κB) activation have been postulated [23].

Whereas the role of $P2Y_1$ receptors in cancer is to decrease cellular proliferation in cancers such as melanoma, the role of $P2Y_2$ receptors is usually to increase cell number. Indeed, in most physiological conditions, activation of human $P2Y_2$ receptors causes an increase in cellular proliferation. This is reflected in some cancer types such as melanoma and lung cancer [18,23]; however, in other cancer types such as oesophageal cancer and some colorectal cancers activation of $P2Y_2$ receptors causes a decrease in cellular proliferation [21,22]. This ambiguity has yet to be fully explained but might be due to the

simultaneous activation of different second messenger systems that have opposite effects on cell number, and also differences in the concentration of ATP needed to cause a response. In addition, the tissue of origin of the cancer and the anaplastic nature of cancers needs to be considered.

P2Y₁₁ receptors

The $P2Y_{11}$ receptor modulates differentiation in the leukaemia-derived cell lines HL-60 and NB4 [41]. In addition to the agonist ATP, ATPγS and BzATP are agonists at this receptor. There are no selective antagonists currently available for $P2Y_{11}$ receptors, although suramin and Reactive blue 2 are effective as nonselective antagonists.

ATP induces the maturation of HL-60 and other acute promelocytic leukaemia cells into neutrophil-like cells [36]. This differentiation arises from a mechanism that activates $P2Y_{11}$ receptors that are coupled to cAMP generation and the activation of protein kinase A.

P2X₅ receptors

The $P2X_5$ receptor also has a role in cell differentiation, and is present in cells in the skin, gut, bladder, thymus, skeletal muscle and the spinal cord [2]. Potent $P2X_5$ receptor agonists are ATP and ATPγS but the only antagonists that are available are the nonselective antagonists suramin and pyridoxalphosphate-6-azophenyl-$2',4'$-disulfonic acid.

Purinoceptors are suggested to have a trophic role in skeletal muscle development and differentiation [42]. $P2X_5$ receptors are present on skeletal muscle satellite cells, and ATP activation of $P2X_5$ receptors inhibits proliferation and stimulates markers of cell differentiation.

The trophic role of the $P2X_5$ receptor in epidermal homeostasis is the regulation of cell differentiation [43]. Indeed, $P2X_5$ receptors are colocalized with markers of cell differentiation, and ATPγS, a potent agonist of $P2X_5$ receptors, decreases keratinocyte number. This is thought to be due to an ATPγS-mediated change in the keratinocyte cell cycle to a state where these cells are differentiating and can no longer proliferate. Extrapolating this principle to cancer cells, $P2X_5$ receptors have been identified in squamous cell carcinomas of the skin and prostate cancers [19,44]. ATP and its analogues acting on these cells cause a decrease in cell number, which might be due to a change in the cell cycle from proliferation to differentiation.

P2X₇ receptors

The $P2X_7$ receptor differs in that, in addition to functioning like other P2X receptors, when exposed at high concentration or for a long period to ATP, its cation channel can be converted to a large nonselective transmembrane pore that allows the passage of not only cations but small molecules up to 900 daltons in size [4]. This effect is associated with apoptosis mediated through the caspase enzyme system [45] and the release of interleukin 1β [46]. The idea of a cell-membrane receptor linked to apoptosis is an attractive target for cancer therapy. $P2X_7$ receptors have so far been described in at least five

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Chemical names

KN62: 1-[N,O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine
MRS2179: N^6-methyl-$2'$deoxyadenosine $3',5'$-bisphosphate

different types of cancer [17,19,20,23,27] and, using markers such as TUNEL and caspase enzyme activity assays, have been shown to be functional and mediate an anticancer effect via apoptosis. Pharmacological characterization of these cancer cells with ATP or analogues such as BzATP, which has some specificity for the $P2X_7$ receptor, and specific agonists such as KN62 have demonstrated a reversible decrease in cell number mediated through apoptosis [17].

P2 receptor-mediated signalling pathways

Several signalling pathways and second messenger systems have been suggested as being coupled to P2 receptor signalling (Table 1 and Figure 1). The most common signalling pathway described is an increase in PLC activity, leading to $Ins(1,4,5)P_3$ mobilization and the release of Ca^{2+} from the endoplasmic reticulum. This increase in the intracellular level of Ca^{2+} triggers changes in cell proliferation. 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 receptors, is also being increasingly described in cancer cell lines. Other second messenger systems have been linked to purinoceptors and the number of pathways implicated is likely to continue to increase.

Conclusions and therapeutic potential

Taken together, these results indicate that extracellular nucleotides (purines and pyrimidines) can regulate proliferation, differentiation and apoptosis of cancer cells through different P2 receptor subtypes. Activation of the metabotropic $P2Y_2$ receptor subtype can lead to an increase in cell number in most cancer types whereas activation of the metabotropic $P2Y_1$ and ionotropic $P2X_5$ and $P2X_7$ receptor subtypes leads to a decrease in cell number. The possible modes of action of P2 receptors on cancer cells are summarised in Figure 1.

It has been demonstrated clearly that different receptor subtypes can be present on the same cell and that the varying receptors can have opposite effects on cell number. This arrangement suggests that the control of cell proliferation by extracellular nucleotides might be regulated by a crucial balance of the activities of the receptor subtypes that mediate mitogenic or apoptotic processes.

All the clinical trials to date have used the generic P2 agonist ATP. With the more recent establishment of P2 receptor subtypes and a clearer idea of their individual functions a new avenue of investigation has opened up: targeting P2 receptor subtypes with specific agents rather than ATP. An example of this new strategy would be in the treatment of melanoma by targeting $P2Y_1$ receptors, where 2-MeSADP and MRS2179 are a selective agonist and antagonist, respectively, and $P2X_7$ receptors, where BzATP and the isoquinoline KN62, and coomassie brilliant blue G have potent agonist and antagonist actions, respectively. Several receptor subtype-specific agonists and antagonists have been developed and are either established in clinical practice or are entering trials for a variety of therapeutic functions [3]. These include clopidogrel, a $P2Y_{12}$ receptor antagonist that inhibits platelet aggregation and is used for thrombosis and stroke [47], and diquafosol, a $P2Y_2$ receptor agonist that is used
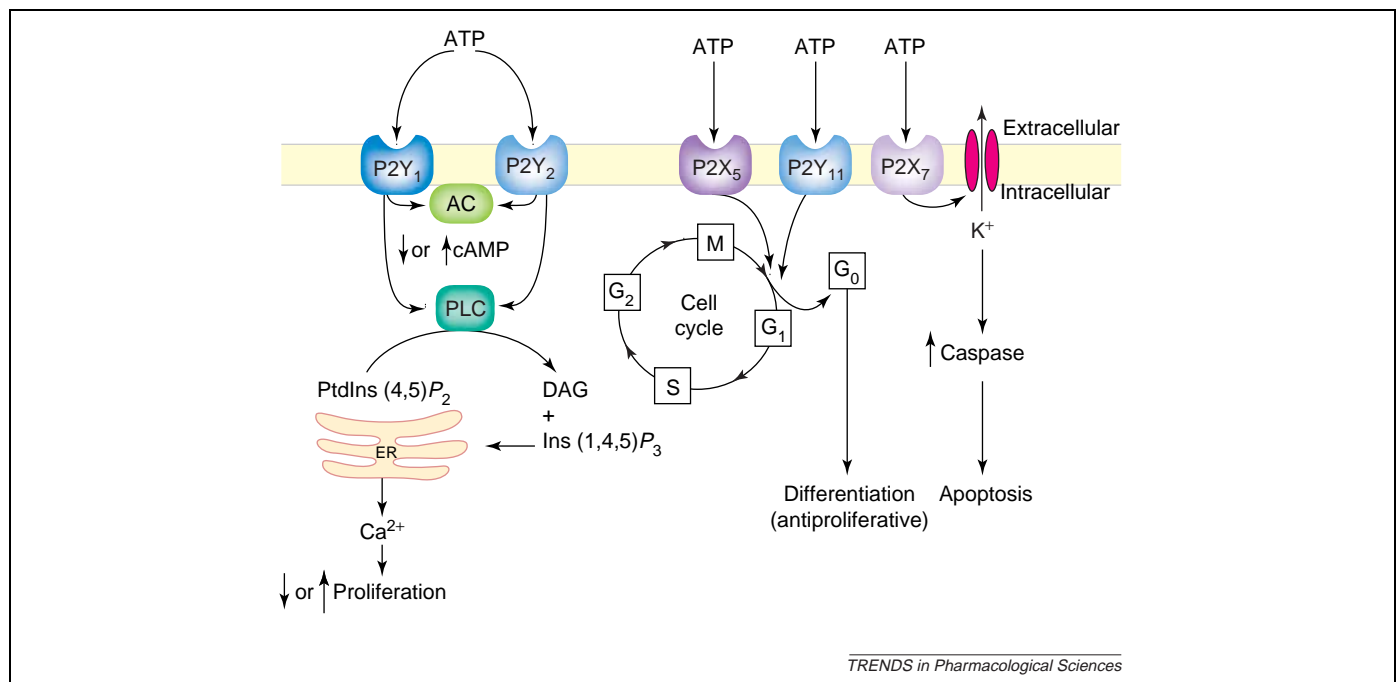
Figure 1. Different mechanisms by which P2 receptor subtypes might alter cancer cell function. $P2Y_1$ and $P2Y_2$ receptors might affect the rate of cell proliferation by modulating adenylyl cyclase (AC) and altering the intracellular levels of cAMP, or by increasing the intracellular level of Ca^{2+} through the phospholipase C (PLC) pathway. $P2X_5$ and $P2Y_{11}$ receptor activation might switch the cell cycle from proliferation into a state of differentiation. The $P2X_7$ receptor activates the apoptotic caspase enzyme system. Abbreviations: DAG, diacylglycerol; $Ins(1,4,5)P_3$, inositol (1,4,5)-trisphosphate; $PtdIns(4,5)P_2$, phosphatidylinositol (4,5)-bisphosphate.

to treat dry eye [48]. Finally, an alternative to using ATP or a receptor subtype-specific compound in isolation as a treatment for cancer would be to study the synergistic effects of these agents when used with established cytotoxic agents.

Research during the past decade has revealed that purinergic signalling might constitute an important and previously unrecognised system that can be targeted as a therapy for cancer. However, P2 receptors are widely distributed throughout the body and the challenge for the future will be to develop agonists and antagonists that are relatively selective for cancer or that can be used in conjunction with other chemotherapy agents.

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