P2 receptors and cancer

Nicholas White and Geoffrey Burnstock

Autonomic Neuroscience Centre, Royal Free and University College Medical School, Rowland Hill Street, London, UK, NW3 2PF

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 vivo 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 Ca²⁺ 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]. P2Y₁, P2Y₂, P2Y₄ and P2Y₆ receptor mRNA has been detected using RT–PCR; however, no protein for the P2Y₄ receptor has been identified using polyclonal antibodies. Agonist and antagonist studies suggest that the P2Y₁ receptor subtype mediates a decrease in cell number whereas the P2Y₂ receptor subtype mediates an increase in cell number. There is no alteration in cell proliferation mediated through activation of P2Y₆ receptors. The alterations in cell number are most likely to be mediated through a PLC-coupled increase in intracellular Ca²⁺ levels. In addition, P2X₇ 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 P2Y₁, P2Y₂, P2X₅ and P2X₇ receptors [19]. Colocalization of P2Y₂, P2X₅ and P2X₇ 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 P2Y₂ receptors and a decrease in cell number via P2X₇ 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 P2Y₂ receptor mRNA was 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 P2Y₂ receptors stimulated cell proliferation, with P2Y₁ and P2X₇ 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]. P2X₄ and P2Y₂ receptor mRNA was found in both the tissue samples and the cell line but only P2Y₂ receptor protein was expressed. There was a functional response to P2Y₂ receptor activation, which resulted in an increase in the intracellular level of Ca²⁺ and a decrease in cell number over time.

In a single lung cancer cell line, A549, the expression of functional P2Y₅ and P2Y₆ 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 P2Y₁ and P2Y₁₂ 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 P2Y₁₂ receptor appears to increase cell number, the P2Y₁ receptor has opposite effects in different cell lines, causing either an increase or a decrease in the cell population.

Functional P2X₇ receptors have been studied intensively in primary cultures of human cerebral 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 P2X₇ 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 deoxycytidine 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 Ca²⁺ [28,29]. More recently, activation of P2Y₂
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 P2X7 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 P2Y2 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 P2X7 and P2Y11 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 P2X7 receptor causes cell death by increasing intracellular levels of Ca\(^{2+}\) and the P2Y11 receptor is coupled to adenyl 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 \(\mu\)g 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

---

**Table 1. P2 receptor subtype expression in different cancers\(^a\)**

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Primary tissue</th>
<th>Cell line</th>
<th>mRNA</th>
<th>Protein</th>
<th>Second messenger system or functional response</th>
<th>Change in cell number</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>Yes</td>
<td>A375</td>
<td>P2X(_2), P2Y(_1), P2Y(_2), P2Y(_4), P2Y(_6)</td>
<td>P2X(_7), P2Y(_1), P2Y(_2), P2Y(_4), P2Y(_6)</td>
<td>Caspase-3,7</td>
<td>↑ P2Y(_2), ↑ P2Y(_7)</td>
<td>[17,18]</td>
</tr>
<tr>
<td>Skin (squamous cell carcinoma)</td>
<td>Yes</td>
<td>A431</td>
<td>Not investigated</td>
<td>P2X(_6), P2X(_7), P2Y(_2), P2Y(_4), P2Y(<em>6), P2Y(</em>{11})</td>
<td>Caspase-3, TUNEL</td>
<td>↑ P2Y(_2), ↑ P2Y(_6)</td>
<td>[19]</td>
</tr>
<tr>
<td>Colorectal</td>
<td>Yes</td>
<td>HT29, HCT8</td>
<td>P2X(_1), P2X(<em>6), P2X(</em>{11}), P2Y(_2), P2Y(_4), P2Y(<em>6), P2Y(</em>{11})</td>
<td>P2X(_1), P2X(_4), P2X(_7), P2Y(_2), P2Y(_4), P2Y(<em>6), P2Y(</em>{11})</td>
<td>Cell death, ELISA</td>
<td>↑ P2Y(_1), ↑ P2Y(_2), ↑ P2Y(_6)</td>
<td>[20,21]</td>
</tr>
<tr>
<td>Oesophageal</td>
<td>Yes</td>
<td>CaCo-2, Kyse-140</td>
<td>P2X(_6), P2X(_7), P2Y(_2), P2Y(_4)</td>
<td>P2Y(_2)</td>
<td>Caspase-3</td>
<td>↑ P2Y(_2)</td>
<td>[22]</td>
</tr>
<tr>
<td>Lung</td>
<td>No</td>
<td>A469</td>
<td>P2Y(_2), P2Y(_6)</td>
<td>Not investigated</td>
<td>CASP-3</td>
<td>↑ P2Y(_2), ↑ P2Y(_6)</td>
<td>[23]</td>
</tr>
<tr>
<td>Prostate</td>
<td>No</td>
<td>PC-3, DU145</td>
<td>P2X(_6), P2X(_9), P2X(_7), P2Y(_2), P2Y(_4), P2Y(<em>6), P2Y(</em>{11})</td>
<td>P2X(_6), P2X(_7), P2Y(_2), P2Y(_4), P2Y(<em>6), P2Y(</em>{11})</td>
<td>PLC-mediated change in [Ca(^{2+})], TUNEL</td>
<td>↑ P2X(_6), ↑ P2Y(_2), ↑ P2Y(_6)</td>
<td>[24–26]</td>
</tr>
<tr>
<td>Brain tumours</td>
<td>No</td>
<td>1321N1, C6</td>
<td>U-251MG, U138-MG, U-87MG</td>
<td>CaSki</td>
<td>Not investigated</td>
<td>P2X(_4), P2X(_5), P2X(_7)</td>
<td>↑ P2X(_2), ↑ P2X(_7)</td>
</tr>
<tr>
<td>Cervical</td>
<td>Yes</td>
<td>MCF-7, Has578T</td>
<td>SK-BR-3, T47-D, MDA-MB-231</td>
<td>Not investigated</td>
<td>P2X(_4), P2X(_7), P2Y(_2), P2Y(_7)</td>
<td>↑ P2Y(_2)</td>
<td>[28–30,50]</td>
</tr>
<tr>
<td>Breast</td>
<td>No</td>
<td>OVCAR-3, EFO-21, EFO-27</td>
<td>HEC-1A, Ishikawa</td>
<td>P2X(_7), P2X(_9)</td>
<td>Not investigated</td>
<td>[Ca(^{2+})], [K(^{-})]</td>
<td>↑ P2Y(_2)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>No</td>
<td>OVCAR-3, EFO-27</td>
<td>EFO-27, HEC-1A, Ishikawa</td>
<td>Ishikawa</td>
<td>P2Y(_2), P2Y(_4), P2Y(<em>6), P2Y(</em>{11})</td>
<td>↑ P2Y(_2), ↑ P2Y(_4), ↑ P2Y(<em>6), ↑ P2Y(</em>{11})</td>
<td>[31–37]</td>
</tr>
<tr>
<td>Endometrial</td>
<td>No</td>
<td>HL-60, Ishikawa</td>
<td>Ishikawa</td>
<td>P2X(_7), P2X(_9), P2Y(_2), P2Y(_4), P2Y(<em>6), P2Y(</em>{11})</td>
<td>↑ P2X(_7), ↑ P2Y(_2), ↑ P2Y(_4), ↑ P2Y(<em>6), ↑ P2Y(</em>{11})</td>
<td>↑ P2X(_7), ↑ P2Y(_2), ↑ P2Y(_4), ↑ P2Y(<em>6), ↑ P2Y(</em>{11})</td>
<td>[34–36]</td>
</tr>
<tr>
<td>Haematological malignancies</td>
<td>Yes</td>
<td>HL-60, Ishikawa, EFO-27, SK-BR-3</td>
<td>Ishikawa</td>
<td>P2X(_7), P2X(_9), P2Y(_2), P2Y(_4), P2Y(<em>6), P2Y(</em>{11})</td>
<td>↑ P2X(_7), ↑ P2Y(_2), ↑ P2Y(_4), ↑ P2Y(<em>6), ↑ P2Y(</em>{11})</td>
<td>↑ P2X(_7), ↑ P2Y(_2), ↑ P2Y(_4), ↑ P2Y(<em>6), ↑ P2Y(</em>{11})</td>
<td>[37–39]</td>
</tr>
<tr>
<td>Bladder (TCC)</td>
<td>No</td>
<td>HT-1376, ARO</td>
<td>Not investigated</td>
<td>P2Y(_1), P2Y(_2)</td>
<td>Not investigated</td>
<td>Not investigated</td>
<td>↑ P2Y(_1), ↑ P2Y(_2), ↑ P2Y(_6)</td>
</tr>
<tr>
<td>Thyroid</td>
<td>No</td>
<td>ARO</td>
<td>Not investigated</td>
<td>P2Y(_1), P2Y(_2), P2Y(_4), P2Y(<em>6), P2Y(</em>{11})</td>
<td>Not investigated</td>
<td>Not investigated</td>
<td>↑ P2Y(_1), ↑ P2Y(_2), ↑ P2Y(_6)</td>
</tr>
</tbody>
</table>

\(^a\)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 deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling.
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 IIIIB (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 P2Y1, P2Y2 and P2Y11 receptors and the ionotropic P2X5 and P2X7 receptors, have been implicated in cancer to date. Alteration of cancer cell number might be due to modulation of cell proliferation via P2Y1 and P2Y2 receptors, stimulation of differentiation with subsequent inhibition of proliferation via P2X5 and P2Y11 receptors or induction of cell death (apoptosis) via P2X7 receptors.

**P2Y1 and P2Y2 receptors**

P2Y1 and P2Y2 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 P2Y1 receptors and uridine 5′-triphosphate (UTP) for P2Y2 receptors, and antagonists such as MRS2179 (see Chemical names) for P2Y1 receptors [4]. The main signal transduction pathway of P2Y1 and P2Y2 receptors is PLC activation, which leads to the formation of inositol (1,4,5)-trisphosphate \([\text{Ins}(1,4,5)P_3]\) 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 P2Y1 receptors in cancer is to decrease cellular proliferation in cancers such as melanoma, the role of P2Y2 receptors is usually to increase cell number. Indeed, in most physiological conditions, activation of human P2Y2 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 P2Y2 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 P2Y11 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 P2Y11 receptors, although suramin and Reactive blue 2 are effective as nonselective antagonists.

ATP induces the maturation of HL-60 and other acute promyelocytic leukaemia cells into neutrophil-like cells [36]. This differentiation arises from a mechanism that activates P2Y11 receptors that are coupled to cAMP generation and the activation of protein kinase A.

**P2X5 receptors**

The P2X5 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 P2X5 receptor agonists are ATP and ATPγS but the only antagonists that are available are the nonselective antagonists suramin and pyridoxalphosphate-6-azophe-nyl-2′,4′-disulfonic acid.

Purinoceptors are suggested to have a trophic role in skeletal muscle development and differentiation [42]. P2X5 receptors are present on skeletal muscle satellite cells, and ATP activation of P2X5 receptors inhibits proliferation and stimulates markers of cell differentiation.

The trophic role of the P2X5 receptor in epidermal homeostasis is the regulation of cell differentiation [43]. Indeed, P2X5 receptors are colocalized with markers of cell differentiation, and ATPγS, a potent agonist of P2X5 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, P2X5 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 P2X7 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. P2X7 receptors have so far been described in at least five
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 P2X7 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 \( \text{Ins}(1,4,5)P_3 \) mobilization and the release of \( \text{Ca}^{2+} \) from the endoplasmic reticulum. This increase in the intracellular level of \( \text{Ca}^{2+} \) triggers changes in cell proliferation. In addition, P2Y receptors couple to adenyly 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 P2Y2 receptor subtype can lead to an increase in cell number in most cancer types whereas activation of the metabotropic P2Y1 and ionotropic P2X5 and P2X7 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 P2Y1 receptors, where 2-MeSADP and MRS2179 are a selective agonist and antagonist, respectively, and P2X7 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 P2Y12 receptor antagonist that inhibits platelet aggregation and is used for thrombosis and stroke [47], and diquafosol, a P2Y2 receptor agonist that is used...
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.

References
7 Vorhoff, T. et al. (2005) Cloning and characterisation of the ecto-nucleotidase NTPDase3 from rat brain: predicted secondary structure and relation to other members of the E-NTPDase family and actin. Purinergic Signalling 1, 259–270
24 Czagowski, R. et al. (2002) ADP-evoked phospholipase C-stimulation and adenylyl cyclase inhibition in glioma C6 cells occur through two distinct nucleotide receptors, P2Y1 and P2Y12. FEBS Lett. 513, 179–183
41 van der Weyden, L. et al. (2000) Pharmacological characterisation of the P2Y1 receptor in stably transfected haematological cell lines. Mol. Cell. Biochem. 213, 75–81
45 Humphreys, B.D. et al. (2000) Stress-activated protein kinase/JNK activation and apoptotic induction by the macrophage P2X7 nucleotide receptor. J. Biol. Chem. 275, 26792–26798
53 Pines, A. et al. (2005) Activation of APE1/Ref-1 is dependent on reactive oxygen species generated after purinergic receptor stimulation by ATP. Nucleic Acids Res. 33, 4379–4394