Pathol. Oncol. Res. (2008) 14:219–231
DOI 10.1007/s12253-008-9071-7

REVIEW

Extracellular ATP and Cancer—An Overview with Special Reference to P2 Purinergic Receptors

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Received: 17 April 2008 / Accepted: 22 May 2008 / Published online: 25 June 2008

Abstract Purinergic signal transduction mechanisms have been appreciated as a complex intercellular signalling network that plays an important regulatory role in both short- and long-term processes in practically every living cell. One of the most intriguing aspects of the field is the participation of ATP and other purine nucleotides in the determination of cell fate and the way they direct cells towards proliferation, differentiation or apoptosis, thereby possibly taking part in promoting or preventing malignant transformation. In this review, following a very brief introduction to the historical aspects of purinergic signalling and a concise overview of the structure of and signal transduction pathways coupled to P2 purinergic receptors, the current theories concerning the possible ways how extracellular ATP can alter the function of tumour cells and the effectiveness of anticancer therapies are discussed, including pharmacological, nutritional, vasoactive and 'anti-antioxidant' actions of the nucleotide. The effects of ATP on animals inoculated with human tumours and on patients with cancer are looked over next, and then an overview of the literature regarding the expression and presumed functions of P2 purinoceptors on tumour cells in vitro is presented, sorted out according to the relevant special clinical fields. The article is closed by reviewing the latest developments in the diagnostic use of P2 purinergic receptors as tumour markers and prognostic factors, while discussing some of the difficulties and pitfalls of the therapeutic use of ATP analogues.

Keywords Extracellular ATP · P2 purinergic receptors · Cancer · Proliferation · Differentiation · Apoptosis

Abbreviations

ATP	adenosine 5′-triphosphate
ADP	adenosine 5′-diphosphate
AMP	adenosine 5′-monophosphate
PLC	phospholipase C
IP3	inositol 1,4,5-trisphosphate
AC	adenylate cyclase
cAMP	cyclic adenosine monophosphate
PKA	protein kinase A
PCR	polymerase chain reaction
mRNA	messenger ribonucleic acid
UTP	uridine 5′-triphosphate
IL	interleukin
TNF	tumour necrosis factor
PSA	prostate specific antigen
AML	acute myelogenous leukaemia
ALL	acute lymphoblastic leukaemia
CML	chronic myelogenous leukaemia
MDS	myelodysplastic syndrome
RyR2	ryanodine receptor type 2

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Introduction

Simultaneously or one after the other, three significant independent functions of adenine compounds have been developed in living cells early in the course of evolution: containing high-energy phosphate groups, adenine com-

pounds have become the major energy stores of cells; as part of adenine nucleotides, they have contributed to the structure of nucleic acids; and as recognized for over three decades, they have served as intercellular signalling molecules. Although as early as 1929, Drury and Szent-Györgyi reported the potent effects of extracellular adenosine compounds on the mammalian heart [1], it was not until the late 1950s and early 1960s that the first studies of ATP as a possible neurotransmitter came to light [2, 3]. After a further decade of investigation of non-adrenergic, non-cholinergic (NANC) neurotransmission, the first comprehensive model of storage, release and action on receptors of ATP during purinergic signal transmission was created by Burnstock [4]. The terms 'P1' and'P2 purinoreceptors' were coined in 1978 [5] and the latter group of cell surface receptors was later subdivided into the ionotropic P2X and the metabotropic P2Y subtypes [6].

Availability of ATP

Although the concentration of extracellular ATP can be increased artificially by giving ATP infusions to a patient, the logical question that arises is if there are natural ligands of purinergic receptors that can activate the purinoceptors on tumour cells in vivo, and how these ligands can be released into the extracellular space. ATP, UTP and UDP-glucose, each being an agonist on different purinergic receptor subtypes, can be released from tumour cells of neuronal and non-neuronal origin, like glioma or astrocytoma cells [7]. It has also been shown that a short stress caused by hypotonic saline induces ATP release from colon cancer cells and this ATP contributes to tumour cell death as an autocrine and paracrine agent [8]. This may play a role in the killing of remaining cancer cells achieved by the washing of the abdominal cavity with distilled water, a method used intraoperatively by surgeons. Purine nucleotides can, furthermore, be released from the cytosol of disintegrating necrotic cells, which are always present in the centre of undervascularized, fast-growing tumours. Moreover, tumour cells contain an increased amount of UTP [9], which underlines the importance of $P2Y_2$ and $P2Y_4$ receptors on the surface of tumour cells, as UTP is a more effective agonist on these purinoreceptor subtypes than ATP.

When ATP appears in the extracellular space in one of the above-mentioned ways, it is quickly broken down by a family of enzymes called ectonucleotidases (ectonucleoside triphosphate diphosphohydrolases, ectonucleotide pyrophosphatases/phosphodiesterases, ecto-5′-nucleotidases, alkaline phosphatases, and ectonucleoside diphosphokinases; for review, see Zimmermann [10]). Most papers focus on expressional and/or functional changes of purinergic signalling in tumours from the aspect of receptors, whereas degrading enzymes of extracellular nucleotides are much less investigated, even though enhancement or suppression of this signalling pathway is equally possible by modifying the activity of the latter. A recent paper by Bavaresco and colleagues supports this idea, inasmuch as they have shown that the beneficial effect of dexamethasone in malignant glioma is at least partly due to the inhibition of the proliferation of C6 rat glioma cells, which may well be the result of the increased expression and activity of ecto-5′-nucleotidase/CD73, an enzyme responsible for the breakdown of ATP and the formation of adenosine, both known to modulate proliferation [11]. An increased expression of the ectonucleotidases CD39 and CD39L1 (accompanied by the upregulation of $P2X_7$, $P2Y_2$, and $P2Y_6$ receptors) was also reported in pancreatic cancer tissues and, moreover, high tissue mRNA levels of CD39 significantly correlated with better long-term survival after tumour resection in patients with pancreatic cancer, underlining the importance of these enzymes [12]. A recent paper of the same group demonstrates that deletion of CD39 in mice results in abrogation of angiogenesis, causing decreased growth of implanted tumours and inhibiting development of pulmonary metastases [13].

As also implied in the previous paragraph, one of the greatest uncertainties as far as the mode of action of ATP in living organisms is concerned is the rapid breakdown of ATP to ADP, AMP and finally adenosine. One must always bear in mind when discussing the pro- or antitumour effects of ATP, that to a (mostly unknown) extent, adenosine can certainly be responsible for the observed effect as it is also known to take part in tumour genesis via P1 receptors [14] (see also Fig. 1).

P2 Purinergic Receptors—Structure and Signal Transduction

P2X receptors are created by the homo- or heteromultimerization of three to six subunits of the $P2X_{1–7}$ subunits, thus there are seven types of homomeric P2X purinoceptors [15]. The subunits consist of 384 to 595 amino acids that form two transmembrane domains and an extracellular loop, with the C- and N-terminals located intracellularly. Following the binding of ATP to the extracellular binding site, a non-selective cation channel that is more permeable to Ca^{2+} than to Na^{+} is opened and the cell is depolarized [16, 17]. The intracellular concentration of Ca^{2+} is therefore increased by the influx of Ca^{2+} ions and as a result of secondary mechanisms following depolarization (Fig. 1).

Some P2X receptors are distinct from other members of this group in their signalling, which bears special relevance to their participation in tumour formation (Fig. 1). Although

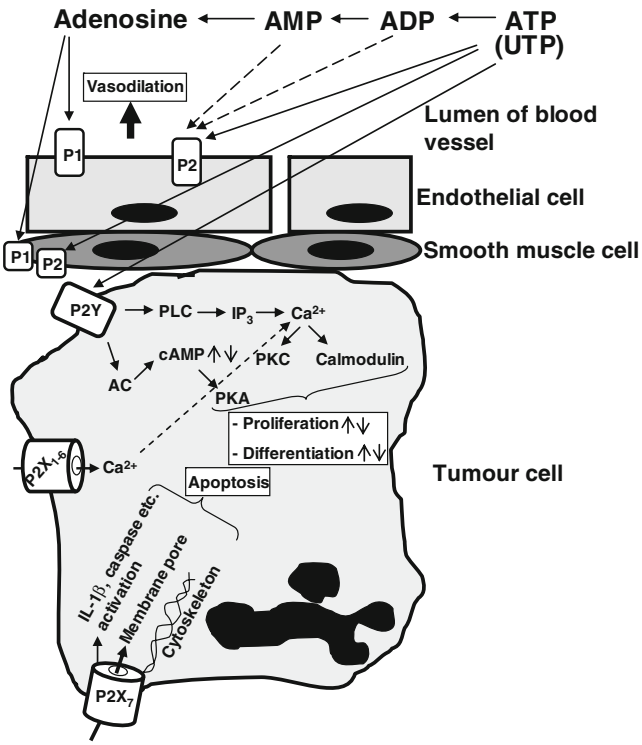

Fig. 1 The effects of P2 receptor activation in tumours. ('X ↑↓' refers to an increase or decrease, respectively, in the concentration or process of X'). Here and in the subsequent figures the charasteristic bizarre, anaplastic nucleus of the tumour cell is depicted as having an amorphous shape

the significance of $P2X_5$ channels being permeable also to Cl^- anions is not clear yet [18], it is now generally accepted that $P2X_7$ receptors play an important role in malignant transformation of several different cell types as will be shown below. This receptor subtype differs from other P2X purinoceptors in that sustained presence of its agonist induces the formation of a large pore in the cell membrane permeable to molecules of up to 900 Da in molecular weight. Besides, as part of a large signalling complex [19], it activates and/or causes the release of numerous molecules such as caspases (caspase-1 [20], caspase-3 [21], caspase-9 [22]), IL-1β [23], kinases and phosphatases [19, 24], partly due to, but also independent of the increased intracellular Ca^{2+} level. The third group of effects that is initiated by the activation of the receptor is the re-organization of cytoskeletal structures which are directly connected to the long intracellular C-terminal 'tail' of the $P2X_7$ receptor through α3-laminin, β2-integrin, β-actin, α-actinin etc. [16], a process that leads to membrane blebbing [24]. $P2X_7$ receptor activation followed by pore forming, enzyme activation and cytoskeletal re-organization results in apoptosis in several cell types (see below), rendering this purinoceptor subtype a target in cancer research for over a decade, initiated by the first reports of the apoptotic effect of the P2Z, now termed $P2X_7$ receptor [25].

P2Y receptors, on the other hand, are G protein coupled, seven-transmembrane-domain metabotropic receptors of 308 to 377 amino acids [26]. So far eight mammalian P2Y receptors have been cloned ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$) [27, 28]. The subtypes that were recognized earliest ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$) are coupled to G_q proteins, activate the phospholipase C (PLC)–inositol 1,4,5-trisphosphate (IP_3) signalling pathway and finally release Ca^{2+} from the intracellular stores (Fig. 1). Besides this signalling mechanism, the other four subtypes also activate ($P2Y_{11}$) [26] or inactivate ($P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$) [29, 30] adenylate cyclase via G_s and G_i proteins, respectively, and the alterations in the cAMP level of the cytoplasm modify the activity of protein kinase A (PKA).

Purinergic receptors are widely distributed [26] and usually more than one subtype can be found on a cell, although the density of a given subtype and the combination of the subtypes varies between tissues as well as the stage of differentiation, as, for instance, demonstrated by work from our laboratory in the case of skeletal muscle [31, 32]. The diversity of purinergic signalling manifests itself in an array of short-term (e.g. neurotransmission, thrombocyte aggregation, nociception etc.) and long-term (e.g. malignant transformation, inflammation, bone resorption, atherosclerosis etc., all via the modification of proliferation, differentiation and apoptosis) effects, reviewed for example by Burnstock [33].

ATP and Tumours—Mode of Action

The effect of ATP on in vitro and in vivo tumour growth was first studied in an animal model by Rapaport in the 1980s [34–36]. Since then clinical trials have also been conducted to test the effect of ATP infusions on patients with cancer (detailed below). Besides, primary tumour cells and cell lines have been tested, too, to prove the presence and in most cases the functioning of purinergic receptors. Despite the fact that purinergic signalling has been studied extensively, the possible anticancer action of ATP should not at all be simplified to this nucleotide acting as an agonist on the members of the purinoreceptor family located in the cell surface membrane.

Pharmacological Effects of ATP Through Dysfunctional or Mislocated Purinergic Receptors

Besides the usual effects exerted through normal purinergic receptors in their typical location detailed in the previous section, dysfunctional P2 receptor proteins may also appear in malignantly transformed cells (Fig. 2), leading to false conclusions if only purinoceptor mRNA or protein expression levels are tested, for example by PCR or immunocyto-

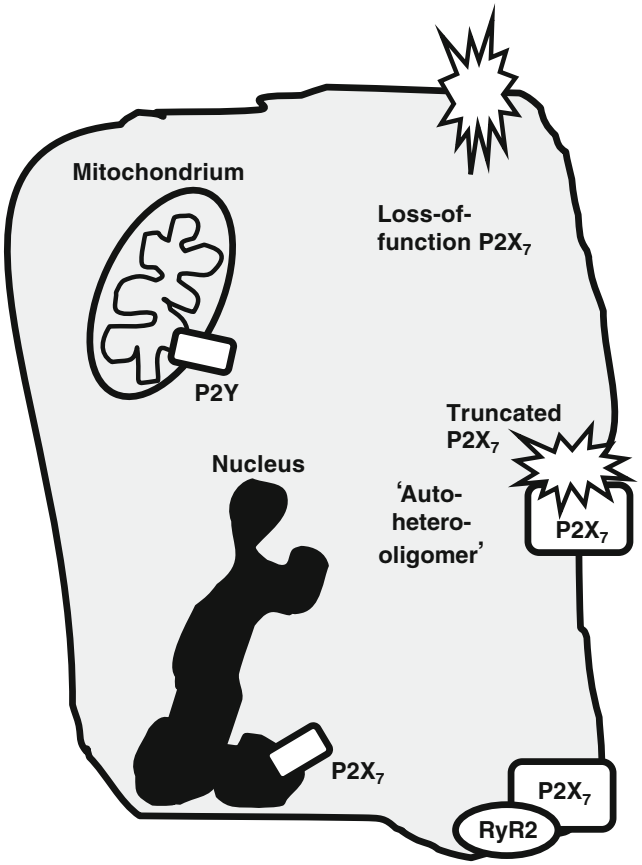

Fig. 2 Dysfunctional or mislocated purinergic receptors in tumour cells. For explanation see text

or -histochemistry. The frequency of the loss-of-function mutant allele A1513C of the $P2X_7$ receptor is three-fold greater in patients with chronic lymphocytic leukaemia than in controls [37]. It was also demonstrated, that even though the expression of the $P2X_7$ receptor is higher in several types of leukaemia, certain $P2X_7$ positive cell lines (J6-1, Namalwa, and LCL-H) fail to display the characteristic functions of this receptor [38]. Sometimes, however, no mutation of the purinergic receptor is found and yet it shows unique characteristics in tumour cells, as we have found in the case of the $P2X_7$ receptor in melanoma cell lines [39]. We have demonstrated that the overexpression of another protein, namely the type 2 ryanodine receptor, modified the function of the purinoceptor and turned it into an antiapoptotic device supposedly through the formation of a functional molecular complex. Similarly, the function of the normal $P2X_7$ subunit may be altered by 'auto-hetero-oligomerization' with the naturally occurring truncated form of the protein that fails to form pores and mediate apoptosis and is upregulated in epithelial cervical cancer cells [40]. Finally, abnormal localization may also cause an altered function of purinergic receptors in tumour cells. Besides localization in the surface membrane, the $P2X_7$ receptor protein has been demonstrated in the nucleus of melanoma cells [39] and $P2Y_1$, $P2Y_2$ and $P2Y_{12}$ receptors in the mitochondria of glioma cells [41], though the functions of these receptors in these subcellular locations are unclear.

Nutritional, Vascular and 'Anti-antioxidant' Effects of ATP

To make the situation even more complex, ATP can influence cancer cells not only pharmacologically by way of its specific cell surface receptors, but also nutritionally, through its vascular effect, and also via depletion of the antioxidant pool in cells (Fig. 3). The nutritional effect of ATP means that the administration of the nucleotide is known to expand the ATP pool of red blood cells [42, 43]

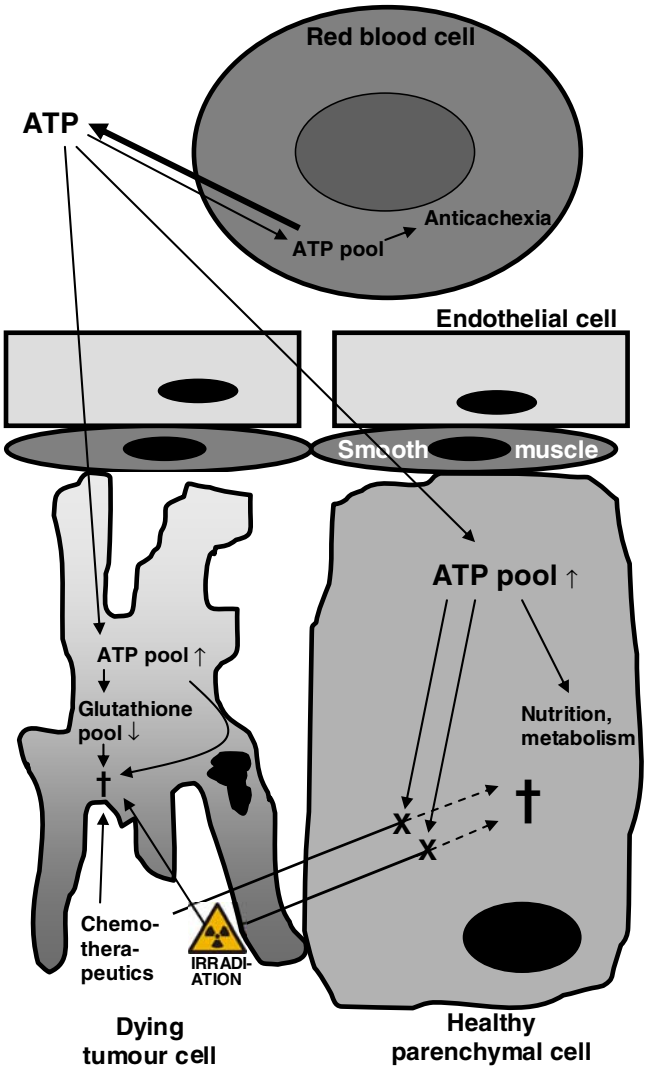

Fig. 3 The function of ATP pools in tumour cells, healthy parenchymal cells and red blood cells ('A ↑' and 'A ↓' refer to an increase or decrease in A, respectively. † denotes the death of the cell. ——x- - - → denotes that the process indicated by the *arrow* is inhibited by the increased ATP pool)

and hepatocytes [43], which may be responsible for the effects attributed to ATP. It is tempting to speculate, that similar ATP pool expansion might occur in other cell types, too—even in the tumour cells themselves. Considering the fact that malignancies have long been associated with increased glycolysis accompanied by impaired oxidative metabolism and decreased availability of ATP [44], supplementation of this molecule may in itself have an antitumoural effect. The vascular aspect of ATP effect originates from the observation of Baba and colleagues that intracarotid administration of ATP selectively increased the blood flow of RG-C6 tumours transplanted into rats, suggesting that ATP might be useful in directing chemotherapeutic drugs towards brain tumour cells [45]. Finally, ATP is known to deplete the glutathione pool in tumour cells, thus making them more susceptible to therapeutically applied oxidative stress induced by chemotherapeutic drugs and/or irradiation [46, 47].

P2 Purinergic Receptors in Tumour Cells and Cell Lines In Vitro

Table 1 summarizes our present knowledge of P2 receptor expression and function in different tumour types, obtained either from the pathological examination of surgical biopsies, or from cultured primary cancer cells or immortalized tumour cell lines (references of this section are also included in the table).

In *epithelial lung cancer cells*, P2 receptors mediate controversial effects of ATP in the sense that both proliferative and cytotoxic effects have been described. Activation of these receptors seemingly supports the effect of the chemotherapeutic drugs cisplatin and etoposide in the cell lines tested.

The two types or *brain tumours* that have been investigated to date demonstrate differences in their P2 receptor expression patterns (C6 glioma cells have $P2Y_{1,2,12}$ receptors, while neuroblastoma cells lack $P2Y_{12}$ receptors but express a wide range of P2X receptors, including $P2X_7$), yet—looking at the effects of these receptors in the table—one has the impression that both tumour types benefit from the presence of purinergic signalling through proliferation, stimulation of neurite outgrowth and protection against serum deprivation). As opposed to this, urological cancer types like *bladder and prostate carcinomas* seem to be inhibited by the activation of the P2 receptors, most of which do not differ from the receptors found on brain tumour cells: anti-proliferative, pro-apoptotic and pro-necrotic effects have been published in several different cell types.

Uterine *cervix and endometrium cancer cells* express the $P2X_7$ receptor and it has a pro-apoptotic effect, but when the truncated form of the receptor is present and forms 'auto-hetero-oligomers' with the wild-type receptor, the effect of receptor activation is inverted and an anti-apoptotic effect is seen in cervical cancer cells. Interestingly, data gained on endometrial, *ovarium and breast cancer cells* suggest, that the effect of the sole $P2Y_2$ receptor is qualitatively the opposite as compared to when it is present in combination with the $P2Y_4$ subtype, namely, an anti-proliferative response of cells upon agonist administration in the first as opposed to a pro-proliferative in the second case. This does not seem to be the case in *gastrointestinal tract tumours*, as the $P2Y_2$ receptor subtype has been found to inhibit proliferation in both the absence (Kyse-140 oesophageal cancer cells) and the presence (colorectal carcinoma cells) of the other P2U receptor. Both P2Y and P2X receptors have been implicated in not only the formation of tumours in, but also the chronic inflammation of the *pancreas*.

Several *leukemia* types and cells from myelodysplastic syndrome patients have also been examined. The $P2X_7$ receptor seems to be active in these cells causing cytoplasmic free Ca^{2+} increase and PLD (phospholipase D) activation, and exerting anti-proliferative effect, but in leukemic cells mutant and loss-of-function $P2X_7$ receptors have also been found.

In *squamous cell carcinoma cells*, three different types of purinoceptors have been identified and in this case the function of the individual subtypes has been determined, too: $P2X_5$ is responsible for differentiation, $P2X_7$ activation causes apoptosis, while $P2Y_2$ agonists induce proliferation. It is clear that the ratio of the different receptor subtypes appearing on the cell surface and the amount of the more or less specific agonists in the vicinity of the cells will determine whether the tumour is able to grow or its cells start dying. *Melanoma malignum* provides another example for conflicting findings: agonists of the $P2X_7$ subtype have been claimed both to promote and to inhibit apoptosis. This controversy may be solved if we assume that the effect exerted by the receptor is altered by the functional interaction with another structure, in this case the type 2 ryanodine receptor that we found overexpressed.

After emphasizing some aspects in connection with certain specific tumour types and looking at the effects of P2 receptors in them, one would like to draw some conclusions as far as the effect of the specific purinergic receptors are concerned. However, one has to realize that this is barely possible. On the one hand, the complexity of the P2 receptor network is amazing and, on the other hand, the effect of the activation of a given purinergic receptor subtype is hard to foresee. The later depends on the tumour type (see for example the effect of $P2Y_2$ receptors in Kyse-140 oesophageal cancer cells and in OVCAR-3 ovarian cancer cells), or often on the cell type within one type of

Table 1 Expression and function of P2 purinergic receptors in various cancer tissues and cells

Special clinical field	Cancer type	Reference	Origin of cell (primary/cell line)	P2 purinoceptor	Effect of P2 receptor agonist
Pulmonology	Epithelial lung cancer	[59]	A549 cells	P2Y2, P2Y6	Pro-proliferative; increased effectivity of cisplatin
		[57]	PC14, A549 cells	P2?	Cytotoxicity (PC14) enhanced effect of etoposide (PC14, A549)
Neurology	Glioma	[64]	C6 cells	P2Y1, P2Y12	PLC activation; adenylate cylase inhibition
		[65]	C6 cells	P2Y2, P2Y12	Pro-proliferative; defence against serum deprivation
		[41]	C6 cells	Mitochondrial P2Y1, P2Y2, P2Y12	?
		[66]	C6 cells	P2Y12	Pro-proliferative
		[67]	U87 MG, U251 MG, and U138 MG cells	P2?	Pro-proliferative
	Neuroblastoma	[68]	SH-SY5Y cells	P2X1, P2X2, P2X4, P2X5, P2X6, P2X7, P2Y1, P2Y2, P2Y4, P2Y6	Induction of differentiation and cell death (P2Y4)
		[69]	Neuro2a cells	P2Y11	Stimulation of neurite outgrowth
		[70]	Primary neuroblastoma tumour samples; ACN, GI-ME-N, HTLA-230, GI-CA-N, LAN-5, LAN-1, SK-N-BE-2, and SH-SY5Y human neuroblastoma cell lines	P2X7	No apoptosis induction; induction of proliferation; substance P release
Urology	Transitional cell cancer of bladder	[58]	HT-1376 cells	P2X4, P2X5, P2Y11	Anti-proliferative
		[49]	HT-1376 cells	P2X5, P2Y11	Anti-proliferative, pro-apoptotic, pro-necrotic
	Prostate carcinoma	[71]	LNCaP, PC-3, DU145 cells	P2Y1, P2Y2, P2Y6, P2Y11, P2X4, P2X5, P2X7 (not every subtype in every cell type)	Anti-proliferative (ATP); pro-apoptotic (BzATP)
		[72]	PC-3 cells	P2X3, P2X5 P2X7, P2Y2	Anti-proliferative; pro-apoptotic
		[62]	Primary prostate tumour samples	P2X7	?
		[61]	Primary prostate tumour samples	P2X1, P2X2, P2X7	?
		[73]	1E8, and 2B4 cells	P2Y?	Cancer cell invasion
		[48, 60]	PC-3, DU-145 prostate cancer cell lines	P2X5, P2Y11	Pro-apoptotic (in vitro and in vivo)
Gynaecology	Cervical cancer	[22]	Primary cultured human ectocervical epithelial cells; CaSki cells	P2X7	Pro-apoptotic
		[40]	Human cervical cancer cells	Truncated P2X7	'Auto-hetero-oligomerization' with P2X7, anti-apoptotic
	Endometrial cancer	[63]	Primary uterine epithelial cancer tissue	P2X7	Pro-apoptotic
		[74]	HEC-1A and Ishikawa endometrial carcinoma cells	P2Y2	Anti-proliferative
	Ovarian cancer	[75]	EFO-21, EFO-27 cells	P2Y2	Anti-proliferative
		[76]	IOSE-29, IOSE-29EC, OVCAR-3 cells	P2U (Y2, Y4)	Pro-proliferative
		[77]	OVCAR-3 cells	?	Pro-proliferative
	Breast cancer	[78]	MCF7, MDA-MB-231 cells	P2?	Anti-proliferative; pro-apoptotic

Table 1 (continued)

Special clinical field	Cancer type	Reference	Origin of cell (primary/cell line)	P2 purinoceptor	Effect of P2 receptor agonist
		[79]	Hs578T, MCF-7, SK-Br3, and T47-D cells	P2U (Y2, Y4)	Pro-proliferative
		[80]	Primary breast cancer tissue	P2X7	None
		[81]	MCF7, MDA-MB-231 cells	?	K^+ efflux
Gastroenterology	Oesophageal cancer	[82]	Kyse-140 cells	P2Y2	Anti-proliferative
	Gastric carcinoma	[83]	HGC-27 cells	?	Anti-proliferative; pro-apoptotic
	Colorectal carcinoma	[84]	Primary colorectal carcinoma cell culture; HT-29 cells	P2U (Y2, Y4)	Anti-proliferative; pro-apoptotic
		[85]	HCT8 and Caco-2 cells	P2X1, P2X3, P2X4, P2X5, P2X6, P2X7, P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12	Pro-apoptotic; pro-proliferative
		[86]	HT-29 cells	P2Y2	Biphasic (increase/decrease) change in extracellular acidification rate (ECAR)
		[87]	HT-29 cells	P2Y4	?
		[88]	Primary colon cancer tissue samples	P2Y2, P2Y4	?
	Pancreatic cancer	[12]	Primary pancreatic cancer tissue samples	P2X7, P2Y2, P2Y6	Chronic inflammation (tissue remodelling, fibrogenesis) + neoplasia
Endocrinology	Thyroid cancer	[89]	ARO cells	P2?	Translocation and neosynthesis of APE1/Ref-1 protein, protection from H_2O_2 induced cell death
		[90]	ARO cells; Primary thyroid tumour samples	P2Y1, P2Y2	Upregulation of Hsp90 and stimulation of cell proliferation
		[91]	FB1 and FB2 cell lines; Human thyroid papillary cancer tissue samples	P2X7	Receptor upregulation; cytoplasmic Ca^{2+} increase, IL-6 secretion
Haematology	Leukaemia subtypes and myelodysplastic syndrome	[92]	L1210 cells	?	Killing of leukaemic cells
		[93]	HL-60 cells	P2Y11	Anti-proliferative; induction of differentiation
		[94]	Primary cells from B-cell chronic lymphocytic leukemia patients	P2X7	Anti-proliferative
		[37]	Primary cells from chronic lymphocytic leukaemia patients	A1513C mutant P2X7	No or decreased apoptotic function
		[38]	Primary cells from acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and myelodysplastic syndrome (MDS) patients; J6-1, Namalwa, LCL-H and other cell lines	P2X7	Cytoplasmic free Ca^{2+} increase (in most P2X7 positive cell types); loss of function (J6-1, Namalwa, and LCL-H cells)
		[95]	K562, Lucena 1 cells	?	Cytotoxic effect
		[96]	F-36P and HL-60 cells	P2X1, P2X4, P2X5, P2X7, P2Y1, P2Y2, P2Y4, P2Y5, P2Y6, P2Y11	G1/G0 arrest of cell cycle (anti-proliferative); pro-apoptotic
		[97]	B-lymphocytes from subjects with chronic lymphocytic leukaemia (CLL)	P2X7	PLD (phospholipase D) activation

Table 1 (continued)

Special clinical field	Cancer type	Reference	Origin of cell (primary/cell line)	P2 purinoceptor	Effect of P2 receptor agonist
Dermatology	Squamous cell carcinoma	[98]	Primary samples from squamous cell carcinomas; A431 cells	P2X5, P2X7, P2Y2	Differentiation (P2X5); pro-apoptotic (P2X7); pro-proliferative (P2Y2)
	Basal cell carcinoma	[98]	Primary samples from basal cell carcinomas	P2X5, P2X7, P2Y2, P2Y4	Differentiation (P2X5)
	Malignant melanoma	[99]	Primary human melanoma tissue samples, A375 cells	P2Y1, P2Y2, P2Y6	Anti-proliferative (P2Y1); Pro-proliferative (P2Y2)
		[100]	Primary human melanoma tissue samples, A375 cells	P2X7	Pro-apoptotic
		[39]	Primary human melanoma tissue samples; HT-199, HT-168-M1, WM-35 cells	P2X7 (in functional interaction with overexpressed ryanodine receptor type 2)	Anti-apoptotic
Non specific (surgery)	Sarcoma	[86]	MCG 101 cells	P2Y2	Increased extracellular acidification rate (ECAR)

tumour (e.g. $P2Y_2$ receptor function in OVCAR-3 versus EFO-21 ovarian cancer cells). We, therefore, would like to refrain from making definite declarations concerning the 'usual' function of certain receptor subtypes in cancer cells and tissues.

In general, however, it can be stated that most of the effects of purinergic signalling commonly examined are related to the regulation of apoptosis, proliferation and differentiation (but again, there are other examples as demonstrated by the table, e.g. the equally significant invasivity of malignant cells, assessed by Chen and colleagues [73] in the case of prostate cancer), and it is usually supposed that the deregulation of one or more of these three functions is an important contribution to malignant transformation. The most widely accepted view attributes pro-apoptotic function to the *$P2X_7$ receptor*, but even this is challenged by observations displaying different, or sometimes even right the opposite activity of this receptor (see in Table 1, e.g. the results of Raffaghello and colleagues [70] with neuroblastoma cells, those of Wiley and colleagues [37] with leukaemia cells, or our own work [39] with melanoma malignum cells). Another cautious generalization that one might attempt looking at the data of Table 1 is that *$P2Y_1$* and *$P2Y_2$ receptors* tend to regulate cell proliferation in a positive or negative way, depending again on the cell type. Finally, we point out that in both cases when the *$P2Y_{11}$ receptor* seems to exert an effect in solo (in case of HL-60 leukaemia cells and neuro2a neuroblastoma cells), experimental data indicate that this receptor subtype may be responsible for inducing re-differentiation of the already partially dedifferentiated tumour cells.

Effect of ATP In Vivo—Animal Models and Clinical Studies

Testing the Effect of ATP on Animals with Implanted Human Tumours

Following the observation of the anti-tumour effect of ATP in monolayer cultures of human cancer cells [34, 35], Rapaport and colleagues successfully treated CT26 colon adenocarcinoma and CAPAN-1 human pancreatic adenocarcinoma implanted into mice by the intraperitoneal administration of adenine nucleotides [36, 42]. Daily intraperitoneal injections of 1 ml of 50 mM AMP, ADP or ATP yielded elevated blood and plasma levels of ATP of 1 to 5 μM lasting for several hours. The above treatment applied for 10 days resulted in the inhibition of tumour growth and a few "cures" in animals with low tumour burdens. The treatment was not toxic to the host as determined by changes in body weights. Weight loss observed in animals upon progression of the aggressive and fast-growing CT26 tumours was slowed markedly, that is, the anti-cachexia effect of the treatment became obvious. Sustained elevation of ATP levels in the plasma is necessary for the beneficiary effect to be exerted but is difficult to achieve due to the rapid break-down of ATP by ectonucleotidases. Another study demonstrated that elevated plasma ATP levels could be explained by the buffering effect of the expansion of erythrocyte ATP pools preceded by and originating in the enhanced turnover of expanded liver ATP pools following intraperitoneal AMP or ATP injections [43].

The studies of Estrela and colleagues pointed out that ATP-induced tumour growth inhibition is accompanied by a

selective decrease in the content of glutathione within the cancer cells in vivo, which sensitizes tumour cells to chemo- and radiation therapy [46]. They reported that administration of ATP in combination with diethylmaleate and X-rays led to complete regression of 95% of Ehrlich ascites tumours in mice, whereas 1 mmol/kg per day of the nucleotide, when combined with recombinant human tumour necrosis factor-α (rh TNF-α) administration, afforded a 61% inhibition of tumour growth and resulted in a significant extension of host survival [46, 47].

Recently, further implanted tumours of different origins with advanced urological malignancy have also been tested. Following their in vitro studies, Shabbir and colleagues have demonstrated the beneficial anti-tumour effect of daily intraperitoneal injections of ATP on two different types of hormone-refractory prostate carcinoma cell lines (PC-3, DU-145) that had been implanted into male nude athymic mice [48]. Significant (approximately 60% to 70%) reduction was seen in the growth of both freshly implanted and established tumours without any adverse effects on the host mice. They also found that intraperitoneal injections of ATP significantly reduced the growth of implanted bladder cancer (HT-1376, human grade 3 transitional cell carcinoma) cells by a combination of apoptosis and necrosis in athymic mice [49].

Clinical Trials

Although extracellular ATP has been reported to inhibit the growth of a variety of human tumours, to induce resistance of non-malignant tissues to chemo- and radiation therapy, and to have pronounced anticachexia effects [50], its possible use in clinical practice is rather controversial due to the contradictory results of the clinical trials carried out so far. Our present knowledge is basically confined to experiences with patients suffering from advanced (stage IIIB and IV) non-small-cell lung cancer, who were the subjects of the small number of studies attempted to date. The first phase I study to determine the toxicity and maximum safely tolerated dose indicated that a 96-h 50 μg/kg/min infusion is a safe and tolerable dose, and the most common side-effect turned out to be a cardiopulmonary reaction characterized by chest tightness and dyspnea, occurring in more than 36% of the patients at the lowest drug dose used and more frequently at higher doses [51]. Phase II and III studies, however, produced more controversial results. Whereas one trial concluded that intravenous ATP was an inactive agent and caused no objective complete or partial responses [52], another group reported to have proved that ATP had beneficial effects on weight, muscle strength, and quality of life in patients with advanced non-small-cell lung cancer [53], partly due to altering glucose turnover and gluconeogenesis [54] and increasing liver energy status [55]. Nevertheless, even this group found that the ten intravenous 30-h ATP infusions every 2–4 weeks over a 24-week period they applied did not lead to tumour regression, and although the treatment resulted in prolonged survival of the subgroup of weight-losing stage IIIB patients, this was not seen in the case of stage IV or weight-stable patients [56].

While the results of some of the above mentioned clinical trials are promising, both the number of trials and the number of participants in these trials are quite low and do not allow one to make final conclusions, especially taking into consideration the contradictions of the published works. It must be emphasized that these clinical trials deal with non-small-cell lung cancer patients and we are short of results concerning other tumour types. Differences in the protocols used also make comparison difficult. Finally, one should not forget that the above-mentioned trials used ATP in monotherapy, although several of its possible modes of action (as described above) enable this compound to facilitate the effect other chemotherapeutic agents or irradiation. The investigation of these combination therapies has also started, the effect of ATP applied together with etoposide [57], diethylmaleate and X-rays [46], mitomycin C [49, 58], interferon-γ [33], cisplatin [59], mitoxantrone [60] and recombinant human TNF-α [47] is examined, even though these studies are still in the preclinical phase.

Diagnostic and Therapeutic Perspectives

The expanding knowledge already piled up about the effect of ATP and other purines on cancer cells could theoretically be converted into practical use in two ways: purinergic signalling related proteins could be used in diagnostics as tumour markers, and the therapeutic potential of purines or purine analogues could be exploited. As far as the former is concerned, the first efforts have already been made to evaluate the prognostic potential of purinergic receptors. One of the most promising attempts was that of Slater and colleagues who demonstrated that $P2X_1$ and $P2X_2$ purinergic receptors were absent from normal prostate epithelium that did not progress to prostate cancer within 5 years, but in cases when it did, labelling of these two proteins was observed in a stage-specific manner: first in the nucleus, then in the cytoplasm and finally on the apical membrane, as prostate cancer developed [61]. These markers were present up to 5 years before cancer was detectable by the usual morphological criteria as determined by hematoxylin and eosine staining. A similar correlation is present with the $P2X_7$ receptor, with 114 tumours out of 116 staining positively for the receptor at the earliest biopsy, generally

with the less advanced pattern of distribution of immunopositivity [62]. The feasibility of the use of the $P2X_7$ protein as a prognostic factor is underlined by the fact that all patients who exhibited no $P2X_7$ labelling had the level of a commonly used marker of prostate cancer, prostate specific antigen (PSA), smaller than 2, while patients who exhibited stage-specific $P2X_7$ expression, and who later developed obvious prostate cancer, all had a PSA >2 [61].

Another example could be the decreased expressions of the $P2X_7$ receptor mRNA and protein in uterine adenocarcinomas, squamous cell carcinomas and dysplastic lesions as compared to normal endometrial, endocervical, and ectocervical cells, which could constitute the basis of a method of 92% and 100% sensitivity and 100% and 90% specificity (mRNA- and protein-level-based, respectively) to distinguish normal and malignant uterine epithelial tissues [63].

The use of $P2X_7$ receptor mRNA expression as a prognostic factor can be demonstrated nicely in leukaemias as well. Not only were relative expression levels significantly higher in acute myelogenous leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myelogenous leukaemia (CML), and myelodysplastic syndrome (MDS) groups than those in the normal donor group, but after one course of standard induction therapies, the remission rate in the high $P2X_7$ expression group was lower than that in either the $P2X_7$ negative or the low $P2X_7$ expression group, clearly indicating the prognostic potential of determination of the $P2X_7$ mRNA levels in these haematologic diseases [38].

As far as purinergic signalling as a potential anticancer drug target is concerned, we have not reached too far yet. Although the first clinical trials have already taken place, as detailed above, only ATP in monotherapy has been tried. Combined administration of ATP with chemotherapeutics is only at the in vitro stage, as is the use of ATP analogues. Probably the most striking contradiction in this field is that although there is a huge amount of in vitro data on the role of different P2 receptors in cell fate and tumour genesis and progression, results seen in human clinical trials are most often explained by what we termed ‘nutritional, vascular and anti-antioxidant actions’, which are poorly explored on the cellular level. This implies that on the one hand further and more detailed investigation of the latter effects needs to be encouraged. On the other hand, however, we must emphasize as well, that the receptorial effects of the P2 agonist ATP should not be underestimated either. The involvement of P2 receptors in the antitumour actions of ATP infusions is supported by the fact that these infusions saturate plasma ectonucleotidases and are able to maintain sustained micromolar plasma ATP concentrations, just within the specific affinity range of most of the receptors in question.

It would be inevitable to synthetize stable (i.e. resistant to the action of ectonucleotidases in the body) and subtype-specific agonists and antagonists of the purinergic receptors, if selective activation or inhibition of a given function were to be achieved. The task is not easy, first, because the effect of activating P2 receptors on a tumour type is often rather confusing as is clear from Table 1. Second, even if a clearly determined function of a receptor subtype would be defined unambiguously, that is whether an agonist or an antagonist was needed for the desired outcome, purinergic receptors are so wide-spread among different tissues that numerous adverse effects of any drug, no matter how specific for a purinoceptor subtype, should certainly be calculated with. Nevertheless, when discouraged by all these difficulties, one should bear in mind the triumph of the platelet aggregation inhibitor antithrombotic drug clopidogrel, which, though outside the field of oncology, is the clear example of the possible success of selective ($P2Y_{12}$) purinergic receptor antagonists in everyday practice.

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