

Chapter 5

Purinergic Signaling in Glioma Progression



Elizandra Braganhol, Márcia Rosângela Wink, Guido Lenz,
and Ana Maria Oliveira Battastini

Abstract Among the pathological alterations that give tumor cells invasive potential, purinergic signaling is emerging as an important component. Studies performed in *in vitro*, *in vivo* and *ex vivo* glioma models indicate that alterations in the purinergic signaling are involved in the progression of these tumors. Gliomas have low expression of all E-NTPDases, when compared to astrocytes in culture. Nucleotides induce glioma proliferation and ATP, although potentially neurotoxic, does not evoke cytotoxic action on the majority of glioma cells in culture. The importance of extracellular ATP for glioma pathobiology was confirmed by the reduction in glioma tumor size by apyrase, which degrades extracellular ATP to AMP, and the striking increase in tumor size by over-expression of an ecto-enzyme that degrades ATP to ADP, suggesting the effect of extracellular ATP on the tumor growth depends on the nucleotide produced by its degradation. The participation of purinergic receptors on glioma progression, particularly P2X₇, is involved in the resistance to ATP-induced cell death. Although more studies are necessary, the purinergic signaling, including ectonucleotidases and receptors, may be considered as future target for glioma pharmacological or gene therapy.

Keywords ATP · Adenosine · Gliomas · Ectonucleotidases · Animal models of gliomas · E-NTPDases (ectonucleoside triphosphate diphosphohydrolase) · E-NPPs (ectonucleoside pyrophosphatase/phosphodiesterase) · Ecto-5'-nucleotidase/CD73 · P2X₇ · Cancer stem cells

E. Braganhol (✉)

Centro de Ciências Químicas, Farmacêuticas e de Alimentos (CCQFA), Universidade Federal de Pelotas (UFPel),
Campus Capão do Leão S/N Caixa Postal 354, Pelotas CEP 96010900, RS, Brazil
e-mail: e-mail: elizandra.braganhol@ufrgs.br

M. R. Wink

Departamento de Ciências Básicas da Saúde, Universidade Federal de Ciências da Saúde de Porto Alegre,
245 Rua Sarmiento Leite, Porto Alegre CEP 90050-170, RS, Brazil
e-mail: mwink@ufcspa.edu.br

G. Lenz

Departamento de Biofísica, IB e Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul,
9500 Av. Bento Gonçalves, Porto Alegre 61501970, RS, Brazil
e-mail: lenz@ufrgs.br

A. M. O. Battastini

Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul,
2600-anexo Rua Ramiro Barcelos, Porto Alegre CEP 90035-003, RS, Brazil
e-mail: abattastini@gmail.com

Abbreviations

ADA	Adenosine deaminase
ADP	Adenosine diphosphate
Akt	Protein kinase B
ALP	Alkaline phosphatase
AMP	Adenosine monophosphate
APCP	α,β -Methylene ADP
Apyrase	Adenyl-pyrophosphatase
ATP	Adenosine triphosphate
BBB	Brain blood barrier
BBG	Brilliant Blue G
BzATP	2,3-(Benzoyl-4-benzoyl)-ATP
CDK	Cyclin-dependent kinase
CDKN2A (Ink4a/ARF)	Cyclin-dependent kinase inhibitor 2A
CSCs	Cancer stem cell
ECM	Extracellular matrix
Ecto-5'-NT/CD73	Ecto-5'-nucleotidase
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
E-NPP	Ectonucleoside pyrophosphatase/phosphodiesterase
E-NTPDase	Ectonucleoside triphosphate diphosphohydrolase
ERBB2	Human epidermal growth factor receptor 2
ERK	Extracellular signal-regulated kinases
FGF	Fibroblast growth factor
IL-1 β	Interleukin 1 β
IL-6	Interleukin 6
KO	Knockout
MDM2	Murine double minute 2
MET	NF1, neurofibromatosis 1
MMP-9	Metalloproteinase-9
NPCs	Neural precursor cell
NSC	Neural stem cell
OPC	Oligodendrocyte precursor cell
PDGF	Platelet-derived growth factor
PI3K	Phosphatidylinositol 3-kinase
PTEN	Phosphatase and tensin homolog
Ras/MAPK	Ras/Mitogen activated protein kinase
Ras-GAP	Ras-GTPase activating protein
NF1	Neurofibromatosis 1
RB1	Retinoblastoma
CNS	Central nervous system
SVZ	Subventricular zone
TNF- α	Tumor necrosis factor

5.1 Introduction

5.1.1 *Molecular and Cellular Origins of Gliomas*

Two questions are central about the origin of a given cancer: What are the genetic alterations behind formation of this cancer? And in which cells do these alterations find the cellular environment to thrive? Both questions are obviously very difficult to answer and, as in all complex illnesses, there is a wide range of possible answers. This is even more true for cancer, in which every new cancer has its own evolutionary history, but which is constrained by the cellular repertoire and the microenvironment of the tissue in which the cancer develops.

Several landmark papers appeared in the last years aiming at these difficult questions regarding gliomagenesis and now there is a good body of evidence for the most frequent molecular alterations observed in gliomas and also some very convincing evidence on the cells that give rise to gliomas.

As suggested by the multitude of familial cancers, even though the mutation in these cases occurs in all cells, only a few cells eventually progress to form a tumor. Therefore, the cell of origin of mutation was in an ancestor of the afflicted patient, but the cell of origin of the tumor is a cell in the patient. Although in non-familial cancers the mutation may occur in the cell of origin, this is not necessarily the case.

Starting at the end of the 1990s, several studies aimed at identifying the glioma cell of origin, which was based on the expression of oncogenes under the control of promoters supposedly specific for a given set of cells (Visvader 2011). Despite the leakiness of these promoters, the picture that emerged was that glioma cell origin is stem cells, but the exact nature of these stem cells was not clear. Recently, using a mosaic analysis with double markers, Liu et al. (2011) pinpointed the cell of origin as being the oligodendrocyte precursor cell (OPC), at least in a murine model in which gliomas were induced by deletion of p53 and activation of the Ras pathway by deletion of neurofibromatosis 1 (NF1). Interestingly, when these alterations were done in neural stem cell (NSC), tumors also arose, but these were restricted to the OPCs formed from the NSCs. In this case, a multitude of other cells harboring the genetic alterations were formed by the NSCs, but did not produce tumors. This created the concept of cellular environment that allows the development of tumors, clearly differentiating the cell of origin of glioma (OPC), from the cell of mutation, which can potentially be any cell that can give rise to OPC, or OPC itself.

A large analysis of the molecular alterations that drive gliomagenesis revealed that the activation of the Ras/MAPKs and PI3K/Akt pathways, together with the inactivation of p53 are the most common events associated with gliomas.

Oncogenic signals are centered on the activation of Ras/MAPKs and PI3K/Akt, which are mediated by activating genetic alterations in the growth factor receptors EGFR, ERBB2, PDGFRA and MET. Ras activation also occurs through deletion or mutation in the Ras-GAP NF1, while PI3K/Akt activation occurs mainly through deletion and mutation in PTEN, although direct mutations in PI3K was also observed (TCGA 2008).

Among the tumor suppressor pathways inactivated in gliomas, the most prevalent were inactivation of p53, either directly, or through activation of MDM2 or deletion or mutation on CDKN2A (Ink4a/ARF) and inactivation of the CDK inhibitors CDKN2A, 2B and 2C, together with deletion of RB (TCGA 2008). It is important to stress out, however, that these are statistical analysis of the most prominent genetic alterations, and, tumors may arise with genetic alterations other than the one described above.

5.1.2 Glioma Cancer Stem Cells (CSCs)

Since the first histological observation of gliomas, the cytological variability was evident, but only in the last 10 years or so the concept of cancer stem cells was clearly defined and the role of this subtype of cells has been widely studied.

The first identification of glioma CSCs was done by the group of Peter Dirks, which showed that glioma cells expressing CD133 are much more tumorigenic in mice than glioma cell not expressing this cell surface marker (Singh et al. 2003, 2004). Further evidence for the stem like nature of these cells came with the observations that these cells can give rise to functional tumor vasculature, further increasing the complex heterogeneity of glioma tumors (Ricci-Vitiani et al. 2010; Wang et al. 2010).

The importance of CSCs in cancer biology predicts that elimination of this subpopulation of cells, either through induction of cell death or through differentiation should bring a considerable therapeutic benefit. Data from animal models suggest that elimination of CSCs (Vlashi et al. 2009) or the CSC derived endothelial cells of the tumor vasculature (Ricci-Vitiani et al. 2010) is a promising therapeutic strategy. Unfortunately, CSCs are more resistant to radiotherapy due to higher expression of DNA repair enzymes (Bao et al. 2006). On the other hand, chemotherapeutic agents that present the best results in gliomas in humans, such as temozolomide, seems to reduce the proportion of CSCs in the tumors, suggesting that this may be part of the mechanism of action (Beier et al. 2008).

5.1.3 Tumor Microenvironment – Key for Understanding and Targeting Gliomas

A set of genetic alterations in a cell does not guarantee the development of a tumor. Interplay with the tissue microenvironment is a fundamental part of the process of tumorigenesis. Besides being able to form their own blood vessels through differentiation of their CSCs, gliomas also show complex interactions with their neighbor cells and even with the organism. In a breakthrough work, Skog et al. (2008) studied the microvesicles liberated by gliomas in culture and found that these microvesicles contain a set of mRNA enriched in pro-angiogenic genes and that these microvesicles can “transfect” cells. Thus, liberation of microvesicle protected mRNA allows the cancer cells to contribute to the pool of mRNA of their neighbor cells altering their behavior to favor tumor growth. Interestingly, these microvesicles could be observed in the circulations of patients with gliomas, indicating diagnostic potential.

Among the molecules that play important roles in the interplay of gliomas with the microenvironment is glutamate. This molecule, well established as an excitatory aminoacid that is one of the main culprits of neurotoxicity, is secreted by gliomas in rodent models as well as in patients (Buckingham et al. 2011; Marcus et al. 2010; Takano et al. 2001; Ye and Sontheimer 1999).

A recent clinical study with glioma patients using microdialysis technique, found 100-fold higher glutamate concentrations in the tumor resection margin, where the subpopulation of invasive cells is concentrated (Berens and Giese 1999), when compared to the normal peritumoral cortex tissue (Marcus et al. 2010). It has been hypothesized that this peritumoral increasing concentration of glutamate is responsible for the seizures and tumor associated epilepsy, presented by glioma patients as an early and recurrent symptom. The hyper-excitability and epileptic activity of neurons at the peritumoral regions was attributed to glutamate release by gliomas cells via the system x_c -cysteine-glutamate transporter (Buckingham et al. 2011).

Glioma tumors that release glutamate have a substantial growth advantage when compared with those that do not secrete this molecule. The role of glutamate is reinforced by the significantly attenuation on tumor growth by the administration of the NMDA receptor antagonist MK801 (dizocilpine), *in vivo*, but not *in vitro*, confirming the microenvironment rather than cell autonomous nature of the action of glutamate (Takano et al. 2001).

Taken together, these findings point to a model where the glutamate released by gliomas affect the peritumoral microenvironment, causing peritumoral seizures and facilitating the tumor progression by damaging normal tissue. Nevertheless the glutamate by itself can not completely explain how the growing tumor mass causes neuronal cell death along the growing tumor margins. Among the signaling pathways that could synergistically act with glutamate, the purinergic system has emerged as an important candidate.

5.2 Purinergic Signaling in Gliomas

As would be expected for cancer cells, different gliomas express different sets of purinergic receptors (Table 5.1), and only for some their role in glioma biology was studied. As commented at the introduction of this chapter, gliomas originate from oligodendroglial precursor cells (OPCs) and a comparison of the receptors expressed by these cells with several gliomas may help pinpointing the involvement of specific receptors in the OPC to glioma transformation.

From the initial studies of our group we noticed that glioma cells respond in diverse ways to ATP and other purinoceptor agonists and antagonists. ATP and adenosine induce proliferation of several human glioma cell lines such as U138MG, U87MG and U251MG (Morrone et al. 2003). In U138MG cells, the proliferation stimulus was also observed with ADP, UTP, inosine and guanosine treatment suggesting the involvement of the subtypes P2Y₄ and A₃ in this response. Interestingly, ATP and

Table 5.1 Expression of purinergic receptors in gliomas and glial cells

Receptor	Cells expressing	Receptor	Cells expressing
A ₁	OPC (r), Oligo (r), Astrocytes (r), C6 (r), U251MG (h)	P2Y ₁₃	Astrocytes (r), C6 (r), U138MG (h), U251MG (h)
	OPC (r), Oligo (r), Astrocytes (r), C6 (r), GL261 (m), U87MG (h), U138MG (h), U251MG (h)		Astrocytes (r), C6 (r), GL261 (m), U138MG (h)
A _{2A}	OPC (r), Oligo (r), Astrocytes (r), C6 (r), GL261 (m), U87MG (h), U138MG (h), U251MG (h)	P2Y ₁₄	Astrocytes (r), C6 (r), GL261 (m), U138MG (h)
	OPC (r), Oligo (r), Astrocytes (r), U87MG (h), U138MG (h), U251MG (h)		OPC (r), Astrocytes (r), GL261 (m)
A ₃	OPC (r), Oligo (r), Astrocytes (r), C6 (r)	P2X ₂	OPC (r), Astrocytes (r)
	OPC (r), Astrocytes (r), C6 (r), GL261 (m)		OPC (r), Astrocytes (r), C6 (r), GL261 (m)
P2Y ₁	OPC (r), Astrocytes (r), C6 (r), GL261 (m)	P2X ₃	OPC (r), Astrocytes (r), C6 (r), GL261 (m)
	OPC (r), Astrocytes (r), C6 (r)		OPC (r), Astrocytes (r), C6 (r), GL261 (m), U87MG (h), U251MG (h)
P2Y ₂	OPC (r), Astrocytes (r), C6 (r)	P2X ₄	OPC (r), Astrocytes (r), C6 (r), GL261 (m), U87MG (h), U251MG (h)
	OPC (r), Astrocytes (r), C6 (r), GL261 (m), U138MG (h)		Astrocytes (r)
P2Y ₄	OPC (r), Astrocytes (r), C6 (r), GL261 (m), U138MG (h)	P2X ₅	Astrocytes (r)
	Astrocytes (r), U251MG (h), C6 (r)		Astrocytes (r), U87MG (h), U138MG (h)
P2Y ₆	Astrocytes (r), U251MG (h), C6 (r)	P2X ₆	Astrocytes (r), U87MG (h), U138MG (h)
	Astrocytes (r), C6 (r), GL261 (m), U138MG (h), U251MG (h)		OPC (r), Astrocytes (r), GL261 (m), C6 (r), U87MG (h), U251MG (h)

Designations:

Oligo, Oligodendrocytes; OPC, Oligodendrocyte Precursor Cells; (r), Rat; (m), mouse; (h), human; C6, GL261, U251MG, U87MG, U138MG: Glioma cell lines

Data are from Agresti et al. (2005), Verkhratsky et al. (2009) (OPCs and Oligodendrocytes), Braganhol et al. (2008), Ledur et al. (2011), Morrone et al. (2003), Tamajusuku et al. (2010) (Gliomas), Fumagalli et al. (2003), Jacques-Silva et al. (2004a), Lenz et al. (2001), Verkhratsky et al. (2009) (astrocytes)

adenosine also increased the transport of thymidine into the cell, suggesting a much broader effect than the regulation of cell cycle. ATP and adenosine activate the ERK, MAPK and PI3K/Akt pathways, and both pathways are important in mediating the proliferative effects of these purinergic agonists in U138MG human gliomas (Jacques-Silva et al. 2004a). As discussed below, ATP to induce these effects could stem from cell lysis associated with low degradation of ATP in gliomas.

One important aspect of this model is that glioma cells are resistant to ATP induced cell death. ATP, at concentrations above 1 mM is toxic to several cell lines, mostly mediated by the P2X₇ receptor subtype. In neuronal cultures and organotypic cultures of the hippocampus, ATP is toxic at 5 mM (Morrone et al. 2005). Glial cells are normally resistant to ATP-induced cell death, and, in astrocytes, high ATP concentrations and activation of P2X₇ actually activates the PI3K/Akt pathway, which normally has an anti-death role (Jacques-Silva et al. 2004b).

Notably, mouse neural progenitor cells (NPCs), which are able to differentiate into neurons, astrocytes and oligodendrocytes are sensitive to extracellular ATP with a pharmacological and molecular profile that suggests the involvement of P2X₇ and NPCs from P2X₇ KO mice were much more resistant to extracellular ATP (Delarasse et al. 2009). On the other hand, OPCs, which originate from NPCs, but are already restricted to the oligodendrocyte lineage and which recent data indicates to be the main cells of origin of gliomas, express functional P2X₇ receptors and suffer cytotoxicity when treated with high doses of BzATP, but not by a long lasting challenge with ATP up to 3 mM (Agresti et al. 2005). Interestingly, mature oligodendrocytes are quite sensitive even to low concentrations of ATP or BzATP (Matute 2008; Verkhratsky et al. 2009). The observation that glioma originate from a cell type resistant to ATP, while their precursor and differentiated cell are sensitive may be indicative of the importance of resistance to ATP cell death for glioma development. However, the observations that some glioma cell lines lost this resistance indicate that this is not an absolute requirement for glioma growth (Tamajusuku et al. 2010).

5.3 Ectonucleotidases

The effects of nucleotides and nucleosides on purinergic receptors are regulated by the action of ectonucleotidases, which includes ectonucleoside triphosphate diphosphohydrolases (E-NTPDases), ectonucleoside pyrophosphatase/phosphodiesterases (E-NPPases), ecto-5'-nucleotidase/CD73 (Ecto-5'-NT/CD73) and alkaline phosphatases (ALP) (Robson et al. 2006; Zimmermann 2001, 2006). These enzymes operate in concert for the complete nucleotide hydrolysis (e.g. ATP) to nucleoside (e.g. adenosine) and represent a powerful manner to control the effects mediated by extracellular purines (Knowles 2011; Yegutkin 2008).

5.3.1 Ectonucleoside Triphosphate Diphosphohydrolases (E-NTPDases)

The occurrence of adenylyl-pyrophosphatases, which splits the two phosphate groups from ATP, was for the first time demonstrated in muscle cells by Lohmann (Lohmann 1928). Later, Meyerhof proposed the name “apyrase” for enzymes that hydrolyse ATP, ADP and other triphospho- and diphosphonucleosides to their equivalent monophosphonucleosides and inorganic phosphate (Meyerhof 1945). Over the years, apyrases were identified in many different organisms such as plants; insects; parasites and mammals, and they were classified as ATP-diphosphohydrolase (EC 3.6.1.5) to distinguish them from intracellular ATPases. Until 1995, there was an apparent confusion about the molecular and kinetic identity of the enzymes responsible for the hydrolysis of extracellular ATP. In that year, a complete review describing the ecto-ATPases, which were identified as E-ATPases in analogy to other ATPases, was published (Plesner 1995). Ecto-ATPases as well as ecto-apyrases (or ecto-ATP

diphosphohydrolases) are ubiquitous enzymes that hydrolyze extracellular nucleoside tri- and/or diphosphate exhibiting E-type ATPase activity. They hydrolyze several purine and pyrimidine nucleoside tri- and diphosphate but not nucleoside monophosphates. They are dependent on millimolar concentration of Ca^{2+} or Mg^{2+} , are insensitive to specific P-type, V-type and F-ATPases and also alkaline phosphatase inhibitors and have an alkaline optimum pH. Ecto-ATPases/ecto-apyrases are glycoproteins anchored by two transmembrane domains, which makes difficult their solubilization and purification (Plesner 1995).

At present, the previously classified as E-type ATPases are identified as NTPDases belonging to the CD39 family. In mammals, at least eight related and homologous enzymes sharing five apyrase-conserved regions (ACRs), named NTPDase1 to 8, have been cloned and characterized: NTPDase1 (CD39, ATPDase, ecto-apyrase or ecto-ATP diphosphohydrolase), NTPDase2 (CD39L1, ecto-ATPase), NTPDase3 (CD39L3, HB6), NTPDase4 (UDPase, LALP70), NTPDase5 (CD39L4, ER-UDPase, PCPH), NTPDase6 (CD39L2), NTPDase7 (LALP1) and NTPDase8 (Robson et al. 2006; Zimmermann 2001).

NTPDase1-3 and 8 share common membrane topography with two transmembrane domains at the N- and C-terminus and a catalytic site facing the extracellular compartment. These E-NTPDase members differ regarding the preferences for nucleotides, while NTPDase1, 3 and 8 hydrolyses nucleoside tri- and diphosphates equally well, NTPDase2 hydrolyses nucleoside triphosphates with a 30-fold preference (Bigonnesse et al. 2004; Chadwick and Frischauf 1998; Heine et al. 1999; Kaczmarek et al. 1996; Kegel et al. 1997; Smith et al. 1998).

NTPDase4-7 present an intracellular localization such as the Golgi apparatus and endoplasmic reticulum (Biederbick et al. 2000; Shi et al. 2001). NTPDase5 and NTPDase6 lack the C-terminal transmembrane domain and are also expressed in the plasma membrane or as secreted enzymes with specificity for the hydrolysis of nucleoside diphosphates (Hicks-Berger et al. 2000; Oses et al. 2004; Mulero et al. 1999, 2000; Murphy-Piedmonte et al. 2005; Yeung et al. 2000).

Several studies have shown the expression of multiple NTPDases in the CNS (Battastini et al. 1991; Braun et al. 2003; Bruno et al. 2002; Nagy et al. 1986; Pinsky et al. 2002; Wang and Guidotti 1999). NTPDase1, the lymphoid cell activation antigen CD39, is associated with endothelium and vascular smooth muscle cells being strongly expressed by microglia (Braun et al. 2000; Zimmermann 2006). In neurons, an apyrase-like enzyme has been characterized (Boeck et al. 2002) and the expression of multiple ectonucleotidase has been described in PC12 cells (Vollmayer et al. 2001). NTPDase2 is the dominant ectonucleotidase expressed by rat astrocytes (Wink et al. 2006) being also identified in immature Schwann cells of the peripheral nervous system, in satellite glia cells of dorsal root ganglia and sympathetic ganglia and in enteric glia (Zimmermann 2006). This enzyme is also expressed in adult mouse hippocampal progenitors (Shukla et al. 2005) and in type B cells of the subventricular zone (SVZ) (Braun et al. 2003), two neurogenic regions of the adult mammalian brain. Chadwick and Frischauf described the tissue distribution of NTPDase3 in brain (Chadwick and Frischauf 1998). In more recent studies, NTPDase3 was identified in the mitochondrial matrix or closely linked to the inner mitochondrial membrane of hypothalamic neurons. It was also found that decrease of NTPDase-activity resulted in significantly decreased mitochondrial respiratory capacity. The authors assumed that hypothalamic neuronal activity especially that of excitatory neurons may be dependent on the activity of mitochondrial NTPDase3 due to the ATPase activity of this enzyme (Belcher et al. 2006; Kiss et al. 2009). The NTPDase8 is very poorly expressed or absent in brain (Bigonnesse et al. 2004).

5.3.2 Ectonucleotide Pyrophosphatase/Phosphodiesterases (E-NPPs)

The second family of enzymes involved in the extracellular nucleotide metabolism is the ectonucleotide pyrophosphatase/phosphodiesterase family (E-NPPs; EC 3.1.4.1). NPP1-3 have been detected in almost all tissues, although individual isoforms are usually confined to specific substructures and/or

cell types (for review see Bollen et al. 2000; Goding et al. 2003). They hydrolyze pyrophosphate or phosphodiester bonds of a variety of extracellular compounds with a broad substrate specificity, including nucleotides, lysophospholipids and choline phosphate esters that may reflect their roles in various physiological and biochemical processes. NPP1 was originally discovered on the surface of mouse B-lymphocytes as the plasma cell differentiation antigen (PC-1) (Takahashi et al. 1970). It is highly expressed in cells from bone and cartilage with intermediate expression in other tissues, including brain capillary endothelium (Goding et al. 2003). It is interesting to observe that NPP1 has not been detected in neurons or glial cells, although it has been detected in rat C6 glioma cells (Grobben et al. 1999). The second member of this family, the NPP2 was discovered as an autocrine motility factor (autotaxin, NPP2a) (Stracke et al. 1992). The expression of NPP2a has been correlated with oligodendrocyte differentiation and in the formation of myelin sheet (Fuss et al. 1997). In addition, rat brain NPP2 is expressed in glial cells of the cerebellum (Goding et al. 2003). NPP3 is a glycoprotein present in a specific subset of glial precursor cells, which expression is dependent on the stage of differentiation (Blass-Kampmann et al. 1997; Deissler et al. 1995b).

5.3.3 *Ecto-Alkaline Phosphatases (ALP)*

The extracellular nucleotides ATP, ADP (and AMP) can also be hydrolyzed by alkaline phosphatases (ALP, EC 3.1.3.1) (Picher et al. 2003). Human ALPs are encoded by four different gene loci and three of the four isozymes are tissue-specific, the intestinal (IALP), placental (PLALP) and germ cell (GCALP). The fourth ALP is the tissue-nonspecific (TNALP) expressed in relatively high amounts in bone, liver and kidney (Martins et al. 2001). This protein co-localize with detergent-resistant and glycolipid-rich membrane subdomains and is important to control signal transduction and membrane trafficking (Bianchi and Spychala 2003; Matsuoka and Ohkubo 2004). Although TNALP is widely distributed in different tissues, information about its physiological function in the brain is limited (Zimmermann 1996). It was established that this ubiquitous enzyme has a specific regional and sub-cellular localization in the brain of adult animals and could be involved in neurotransmission, as it exhibits a neuronal activity-dependent regulation and it is localized in the synaptic cleft of cortical synapses (Fonta et al. 2004). TNALP is expressed in the neural tube in different brain regions during embryonic development indicating its involvement in developmental steps with a potential role in cortical plasticity and brain disorders (Fonta et al. 2005; Narisawa et al. 1994). In addition, it was shown that TNALP is expressed in capillary endothelium of blood–brain barrier (BBB) and may participate in extracellular phosphorylation/dephosphorylation processes, which are involved in the modulation of organic cation transport at BBB (Calhau et al. 2002). Finally, considering that ATP may be hydrolyzed by E-NPPs generating PPi and that it is known that TNALP is able to hydrolyse PPi into Pi (Goding et al. 2003), it is possible that in addition to E-NTPDases, these two enzymes might cooperate to hydrolyze ATP directly to AMP without transient production of ADP in the extracellular milieu. Therefore, the combination of more than one enzyme may acts as important regulator of purinergic receptor activation by removing efficiently P2 agonists.

5.3.4 *Ecto-5'-Nucleotidase (Ecto-5'-NT/CD73)*

The intermediary product of ATP hydrolysis, AMP, can be hydrolyzed by the action of ecto-5'-nucleotidase (ecto-5'-NT/CD73, EC 3.1.3.5), which is a widely distributed enzyme anchored on the outer surface of the plasma membrane via a glycosyl phosphatidylinositol linkage and also co-localizes with detergent resistant and glycolipid-rich membrane subdomains called lipid rafts (Bianchi and

Spychala 2003). It produces nucleosides from non-cyclic nucleoside monophosphates in the extracellular space, being the best-characterized enzymatic source of extracellular adenosine (Zimmermann 1992). Considering that ATP and ADP can exert an inhibitory action on ecto-5'-NT/CD73 (Dornand et al. 1978), this step of extracellular nucleotide metabolism represents a pivotal role by controlling the activation of P2 and P1 receptors (Komoszynski and Wojtczak 1996). Ecto-5'-NT/CD73 is expressed in many different tissues (Zimmermann 1992) being identified in different brain regions either located as a membrane bound ecto-enzyme or as a soluble enzyme with intracellular localization (Bianchi and Spychala 2003, Heymann et al. 1984). In the Central Nervous System (CNS) it was identified in astrocytes, hippocampal mossy fiber terminals, the choroid plexus and vascular endothelium (Zimmermann 2006). The subcellular distribution of ecto-5'-NT/CD73 in the brain cells shows that the bulk of activity is associated with myelin, synaptosomal and microsomal fractions (Heymann et al. 1984). This enzyme has been proposed as a glial marker and indicator for development, regeneration and plasticity (Lie et al. 1999; Zimmermann et al. 1998). Recently, Stanojevic et al. showed a negative correlation between the enzyme activity and the enzyme protein abundance in the synaptic plasma membrane, indicating additional roles than those related to AMP hydrolysis for ecto-5'-NT/CD73 in the synaptic compartment during postnatal brain development (Stanojevic et al. 2011). The results of this study provided direct evidence for the existence of this ecto-enzyme in the presynaptic compartment and suggest that ecto-5'-NT/CD73 may be a part of general scheme of brain development and synapse maturation. In fact, several studies demonstrate that besides its catalytic function, ecto-5'-NT/CD73 is also an important player on cell-cell and cell-extracellular matrix (ECM) interactions, (Spychala 2000; Stochaj et al. 1989) as well as a modulator of T lymphocytes signaling (for review see Resta et al. 1998). The highly variable level of expression of ecto-5'-NT/CD73 in animal tissues and cells suggests that tissue-specific mechanisms control the expression of this enzyme. Indeed, a number of tissue-specific regulatory elements within the ecto-5'-NT/CD73 promoter were identified suggesting that there might be a high level of complexity in the interactions between binding factors of this gene (Hansen et al. 1995; Spychala et al. 1999).

In addition to the action of ecto-5'-NT/CD73, AMP can also be deaminated to inosine monophosphate (IMP) by an AMP deaminase activity. This alternative enzymatic degradation of AMP might constitute a mechanism to control extracellular adenosine formed from ATP breakdown (Cunha and Sebastião 1991; Goldman et al. 2010).

5.4 Ecto-Adenosine Deaminase (Ecto-ADA)

The extracellular adenosine levels are controlled by the uptake of plasma membrane-located adenosine transporters and/or by the action of adenosine deaminase (ADA; EC 3.5.4.4) that produces inosine (Cunha et al. 2000). ADA was originally considered to be cytosolic but it has been found ubiquitously on the surface of many different cell types including brain synaptosomes and, therefore, it can be also considered an ecto-enzyme (ecto-ADA) (Franco et al. 1997).

Ecto-ADA has an extra-enzymatic function via its interaction with CD26 and other cell-surface proteins (Franco et al. 1998). Ecto-ADA was identified in neuronal populations in the brain of different mammals (Yamamoto et al. 1987). It was also shown that ecto-ADA can be associated with A₁ adenosine receptors (A₁Rs) in different cell types including pig brain cortical membranes (for review see Zimmermann 1996). Accordingly, the co-localization and interaction between A₁Rs and ecto-ADA may be the functional basis of the extra-enzymatic role of ecto-ADA in modulating ligand-induced signaling, desensitization and internalization of A₁Rs (Beraudi et al. 2003; Ciruela et al. 1996; Gines et al. 2001; Saura et al. 1998). Inosine, product of adenosine deamination, was originally thought to have no biological effects, but today it is well established that this nucleoside may participate actively in many biological processes including immunomodulation and neuroprotection. Inosine

preserves the viability of glial cells and neuronal cells during hypoxia and stimulates axonal re-growth after injury. The actions of inosine might involve effects on adenosine receptors, but it is also possible that inosine augments extracellular adenosine levels by competing with the nucleoside transporters and thus generating secondary effects to adenosine binding to its receptors (for review see Haskó et al. 2004). Recently, it was shown that inosine exerts anticonvulsant effect against hyperactivity of the glutamatergic system independently of benzodiazepines or adenosine receptors activation (Ganzella et al. 2011). Thus, besides its non-enzymatic functions, the final step of purine metabolizing cascade catalyzed by ecto-ADA is very important for the control of adenosine availability and inosine production with consequent activation of different signaling pathways.

5.5 Other Ecto-Nucleotide Metabolizing Enzymes

ATP can be also consumed by ecto-enzymes belonging to protein kinase family (ecto-PK, EC 2.7.10-11-12-13) (Ehrlich et al. 1990). These enzymes are located on the surface of normal, transformed or malignant cells (Paas et al. 1999; Redegeld et al. 1999; Seehafer et al. 1984) and the coordinated action of ecto-PK and ALP would participate in the phosphorylation/dephosphorylation mechanisms in a variety of extracellular events, such as cell-cell interaction and transduction of external signals (Calhau et al. 2002; Kübler et al. 1989). Moreover, the presence of purine converting ecto-enzymes such as ecto-nucleotide kinase (adenylate kinase, EC 2.7.4.3) (Nagy et al. 1989), ectonucleoside diphosphate kinase (EC 2.7.4.6) (Yegutkin et al. 2002; Zimmermann 2006) and the identification of ectopic FoF1-ATP synthase on plasma membrane of mammalian cells (Ravera et al. 2011), provide new perspectives for the scenario of extracellular nucleotide metabolism. In summary, the combination of these multiple extracellular ATP-generating systems along with ATP-consuming pathways would complete the panel of enzymatic and non-enzymatic systems involved in the control of availability of specific agonists for nucleotide/nucleoside-selective receptors (Yegutkin et al. 2002).

5.6 Ectonucleotidases in Gliomas

Events that trigger malignant transformation of glial cells into gliomas are poorly understood. Genetic alterations that controls cell proliferation and differentiation, including the regulation in oncogenes expression (MDM2, CDK4, EGFR) and tumor suppressor genes (p53, p16, p15 and RB1) (Louis 1994; Maher et al. 2001; Shapiro 2001; Von Deimling et al. 1995) are common features of glial cell malignant transformation. In addition, glioma progression requires specific microenvironment conditions, which affect tumor cell interactions with neurons, glia and vascular cells in the CNS (Demuth and Berens 2004). Among the pathological alterations that give tumor cells invasive potential, purinergic signaling is emerging as an important component. By activating specific purinergic receptors (P2X and P2Y), extracellular ATP has been shown to mediate events related to cell proliferation, cell differentiation and cell death (White and Burnstock 2006). Nucleotides exert a synergist effect on cell proliferation together with growth factors, chemokines or cytokines (Lemoli et al. 2004). ATP has been identified as a mitogen for v-myc immortalized neural progenitor cells (Ryu et al. 2003). In astrocytes, extracellular ATP regulates ERK function by activating P2Y₁, P2Y₂ or P2Y₄ purinoceptors (Lenz et al. 2000; Neary et al. 2003), indicating the potential for cross-talk with FGF, EGF and PDGF driven cell mitogenic pathways. Adenosine is one of factors that can contribute to tumor progression (Spychala 2000). This nucleoside accumulates at high concentrations in solid tumors and it has been previously shown to stimulate tumor growth and angiogenesis through activation of P1 receptors and to inhibit cytokine synthesis, cell spreading, and adhesion of immune cells to the endothelial wall and the function of T-cells, macrophages and natural killer cells (Spychala 2000).

As described above, the biological effects of nucleotides and nucleosides are regulated by the action of ectonucleotidases, which efficiently control the purinergic receptor activation by hydrolyzing these molecules in the extracellular space. Accumulating evidence suggests that alterations in the extracellular nucleotide/nucleoside metabolism are involved in the growth and progression of gliomas. We demonstrated that glioma cell lines have altered extracellular ATP, ADP and AMP catabolism when compared to astrocytes, showing low rates of extracellular ATP hydrolysis and high rates of extracellular AMP hydrolysis (Wink et al. 2003). C6 glioma presents a low mRNA expression of NTPDases1-6 (Morrone et al. 2006) and human glioma cell lines as well (unpublished data). These results were confirmed by applying a C6 *ex vivo* glioma model, a primary glioma culture obtained directly from rat biopsy specimens that were previously implanted, indicating that the disruption of purinergic signaling is a feature shown not only by glioma cell lineages, but also by *ex vivo* glioma cultures which represent a closer model of original tumors (Braganhol et al. 2008).

In addition, it seems also important to consider the NTPDase5 mRNA expression in C6 cells (Morrone et al. 2006) and in other different glioma cell lines (unpublished results). In addition to the participation of NTPDase5 in the process of re-glucosylation involved in glycoprotein folding in the endoplasmic reticulum (Tombetta and Helenius 1999), an unexpected role of NTPDase5 in oncogenesis was recently revealed (Villar et al. 2007). NTPDase5 was shown to be identical to the PCPH gene, a human proto-oncogene product expressed in human tumor cell lines (Paez et al. 2001, Rouzaut et al. 2001). The neoplastic transforming activity of the NTPDase5/PCPH oncoprotein is mediated by its ability to promote a Ras-independent, sustained activation of ERK (Recio et al. 2000) and/or render cancer cells resistant to a variety of apoptosis-inducing stimuli (Recio et al. 2000; Velasco et al. 1999), including serum deprivation, hyperthermia, ionizing radiation and chemotherapeutic drugs. The resistance to various stress stimuli elicited by NTPDase5/PCPH was mediated by its ability to hydrolyze ATP, decreasing the phosphate donor availability for the kinases involved in the stress-induced phosphorylation cascades with which it interacts (Recio et al. 2002).

As presented before, extracellular degradation of ATP proceeds by a cascade of cell surface-bound enzymes that also includes the E-NPPs and ALP. Catalysis by E-NPPs affects processes as diverse as cell proliferation and motility, angiogenesis, bone mineralization and digestion. In addition, E-NPPs are also implicated in the pathophysiology of cancer, insulin resistance and calcification diseases (Stefan et al. 2006). NPP1 and -3 are expressed in rat C6 glioma cells where they are responsible for the hydrolysis of low extracellular ATP concentration (1–10 μM) (Grobben et al. 1999; Joseph et al. 2004). Moreover, there is emerging evidence for non-catalytic functions of NPPs in cell signaling. For example, a role of NPP3 in tumor transformation was demonstrated by showing that its expression in glioma cells induced morphological changes and enhanced tumor invasive properties (Deissler et al. 1995a). The role of NPP1 expression in C6 glioma cell motility and invasion remains to be determined. Regarding the involvement of ALP in the tumorigenic process, there are a few studies characterizing its participation in glioma biology. ALP activity is restricted to the capillary wall, being stronger in capillaries from glioblastomas than astrocytomas. Moreover, in opposite to normal cells, the ALP activity in glioblastoma is markedly positive on the luminal surface of plasma membrane of endothelial cells. Phosphatase activities in brain tumor appear to change in localization pattern in association with glioma malignancy, which may reflect a higher permeability of the BBB (Maeda et al. 1985). ALP can also contribute to adenosine production from AMP as a substrate, as demonstrated for the neuroblastoma glioma hybrid NG108-15 cells (Ohkubo et al. 2000).

The AMP produced in the extracellular space is hydrolyzed to adenosine by the action of ecto-5'-NT/CD73 (Zimmermann 1992). A large body of evidence suggests that ecto-5'-NT/CD73 has tumor-promotion functions (Spychala 2000). High ecto-5'-NT/CD73 expression levels have been reported in many human solid tumors, such as colon, lung, pancreas, ovary (Su et al. 2001), melanomas (Sadej et al. 2006), breast carcinomas (Zhi et al. 2007) and glioblastoma cells (Ludwig et al. 1999; Wink et al. 2003), being its expression correlated with shorter patient survival time (Spychala 2000). Adenosine, the product of ecto-5'-NT/CD73 activity, has been reported as mediator of cell

proliferation, angiogenesis and may suppress the anticancer immune response (Gessi et al. 2011; Morrone et al. 2003; Spychala 2000). Adenosine concentrations increase within hypoxic regions of solid tumors, including gliomas (Melani et al. 2003), and it has been recognized to interfere with the recognition of tumor cells by cytolytic effectors cells of the immune system (Blay et al. 1997; Merighi et al. 2003). In particular A_{2A} adenosine receptors on T-cell surface may play an immunosuppressive role in large solid tumors, inhibiting incoming antitumor cytotoxic T lymphocytes from destroying the tumor (Koshiba et al. 1997). The immunosuppressive role and the ability to protect against ischemia suggest that A_{2A} activation improves hypoxic tumor cell survival and immune escaping. A_{2B} adenosine receptors seem to contribute to tumor growth, neovascularization and for the release of a subset of cytokines (Gessi et al. 2011). In U87MG human glioma cells, adenosine increases IL-6 and IL-8 expression via stimulation of A_{2B} , while the stimulation of A_3 receptors induced an increase of metalloproteinase-9 (MMP-9) levels, which was responsible for an increase of glioblastoma cells invasion (Gessi et al. 2010). In a parallel investigation, adenosine promoted an increase in U138MG glioma cell adhesion, which was prevented by adenosine receptor antagonists and dipyridamole, indicating the participation of extra- and intracellular signaling pathways in cell adhesion mediated by this nucleoside (Cappellari et al. 2011).

In addition to produce the pro-tumor nucleoside adenosine, ecto-5'-NT/CD73 has a role in the control of cell growth, maturation, differentiation, cell-cell and cell-matrix interactions (Navaro et al. 1998; Turnay et al. 1989; Vogel et al. 1991; Zhou et al. 2007). Notably, increasing cell confluency and culture times led to an increase in ecto-5'-NT/CD73 activity and expression in different glioma cell lines (Bavaresco et al. 2008). The ECM laminin and chondroitin sulfate modulated the ecto-5'-NT/CD73 activity and glioma adhesion in a parallel manner, suggesting the involvement of purinergic signaling in the effects mediated by ECM components (Cappellari et al. 2011). In line to the role of ecto-5'-NT/CD73 and its product adenosine on tumor-promotion actions, the treatment with APCP, a synthetic inhibitor of this enzyme, and AMP significantly reduced glioma cell proliferation (Bavaresco et al. 2008). Additionally, it was shown that the inhibitory effect of quercetin, dexamethasone and indomethacin on glioma cell proliferation was related to the modulation of ecto-5'-NT/CD73 activity (Bavaresco et al. 2007; Braganhol et al. 2007; Bernardi et al. 2007) and the expression of A_3 adenosine receptor mediates the adenosine cell death actions (Gessi et al. 2011). In conclusion, ecto-5'-NT/CD73 expression is important in the glioma development for different reasons: (a) providing the major source of extracellular adenosine and/or (b) interacting directly with extracellular proteins acting as an adhesion molecule.

5.7 The Purinergic Hypothesis of Glioma Invasion

Previous studies from our group indicate a strong involvement of purinergic signaling in the growth and progression of gliomas. The majority of glioma cell lines present an extracellular nucleotide metabolism that has low ATPase and high AMPase activity, with is strikingly different from normal astrocytes, which have high ATPase and low AMPase activity (Wink et al. 2003).

Adenine nucleotides induce cell proliferation in diverse human glioma cell lines (Morrone et al. 2003) and the majority of glioma cell lines are resistant to cell death induced by cytotoxic ATP concentrations (Morrone et al. 2005). As ATP is poorly hydrolyzed by glioma cells and, in addition, can be ectopically produced in C6 glioma plasma membrane (Ravera et al. 2011), this nucleotide could potentially accumulate within tumor, resulting in glioma cell proliferation and neuronal toxicity. We have proposed that besides the glutamatergic system (Takano et al. 2001), the purinergic signaling could also be involved in this process (Morrone et al. 2003, 2005; Wink et al. 2003). In this model, we hypothesize that neuronal death induced by glutamate released from gliomas results in the liberation of ATP and glutamate (normally present in high concentrations in the intracellular milieu) to the

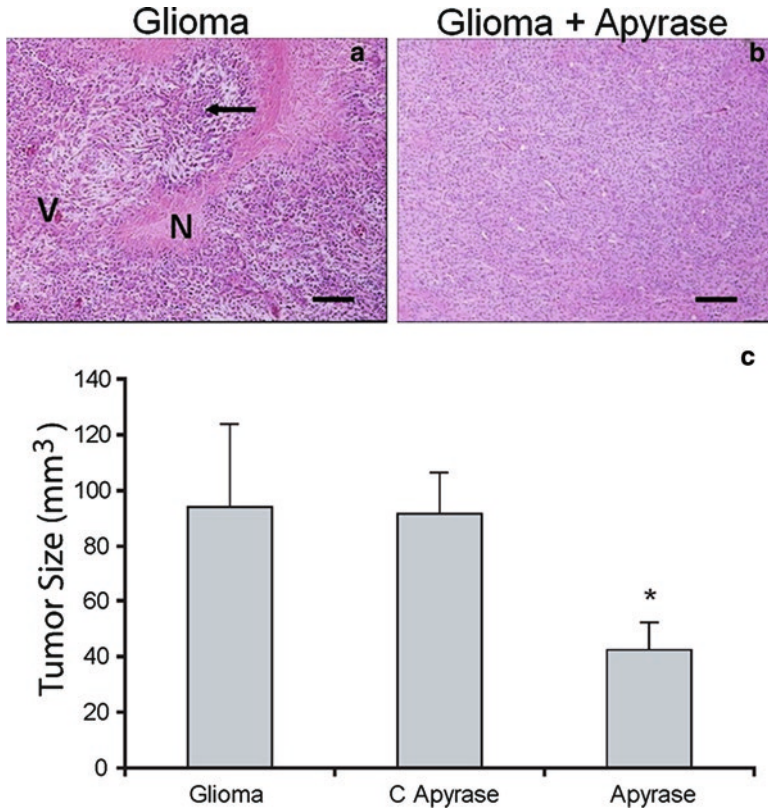


Fig. 5.1 *In vivo* glioblastoma growth is reduced by apyrase co-injection. To determine the effect of apyrase co-injection on *in vivo* glioma growth, C6 cells (1×10^6) were implanted in the right striatum of Wistar rat by stereotaxical surgery in presence (Glioma + Apyrase) or absence of apyrase (Glioma). (a, b) Histological characteristics that define glioblastoma multiforme as seen in rats implanted with gliomas and in rats co-injected with apyrase. Scale bars = 100 μ m. (c) Tumor size quantification of implanted gliomas. Tumor size was evaluated 20 days following glioma implantation. Data represent the mean \pm SD of at least six animals per group. Mean \pm S.E.M. * $p < 0.05$ for comparison versus control, as determined by ANOVA, followed by Tukey-Kramer test. Glioma, rats implanted with C6 cells; CApyrase, apyrase denatured by boiling and co-injected with C6 cells; Apyrase, apyrase (2 U) co-injected with C6 cells; N, necrosis and microvascular proliferation; V, giant cell formation and nuclear pleomorphism (arrow) (Adapted from Morrone et al. (2006). With permission from BioMed Central)

extracellular space leading to more neuronal cell death and glioma proliferation in a positive feedback cycle. Because gliomas, contrary to astrocytes in culture, exhibit low NTPDase expression and activity (Morrone et al. 2006) this feedback is not blocked.

To test this hypothesis, we examined the effect of co-injection of apyrase, an ATP/ADP scavenger, in a C6 rat glioma experimental model, which has been extensively used to test antitumor interventions (Takano et al. 2001). The implanted glioma co-injected with apyrase produced smaller tumors when compared with the rats injected only with gliomas or with gliomas plus inactivated apyrase. According to the pathological analysis, the malignant gliomas induced by C6 co-injected with apyrase exhibited a significant reduction in the mitotic index, necrosis and vascular proliferation, pathological characteristics that indicate a less invasive/proliferative tumor (Fig. 5.1). Considering that the injection of apyrase was done only at the moment of implantation, the effect of ATP/ADP depletion is important probably at the implantation and initial growth of the glioma. This could be of therapeutic interest, because the application of apyrase in the surgical resection cavity could be helpful in reduc-

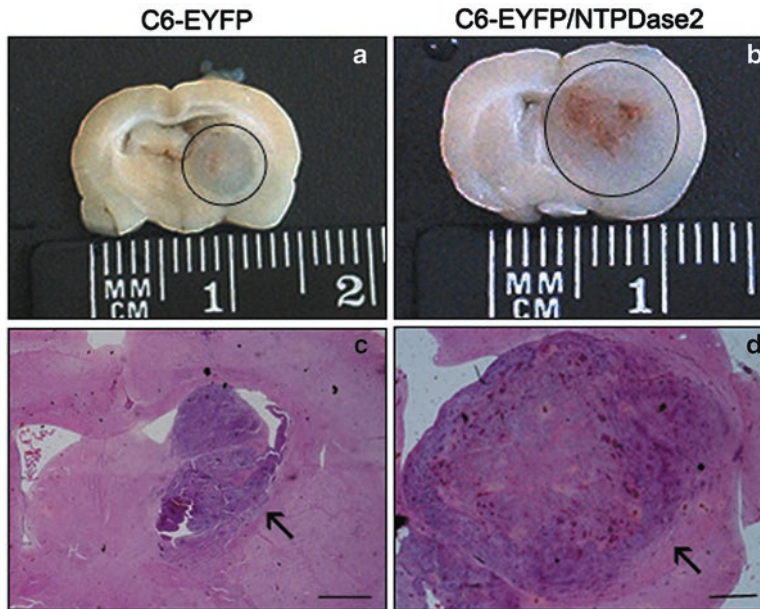


Fig. 5.2 NTPDase2 expression stimulates *in vivo* glioblastoma growth. To determine the glioma growth *in vivo*, equal amounts of C6, C6-EYFP, or C6-EYFP/NTPDase2 cells (1×10^6 cells) were implanted in the right striatum of Wistar rat brains by stereotaxical surgery. The animals were killed 20 days later and glioma sections were dissected and analyzed for tumor growth. (a, b) Photographs of rat brain slices of C6-EYFP and C6-NTPDase2-implanted gliomas. The gliomas are marked with a circle. (c, d) Representative sections of C6-EYFP and C6-EYFP/NTPDase2-implanted gliomas stained with HE. Scale bars=0.5 mm (Adapted from Braganhol et al. 2009. With permission from John Wiley and Sons)

ing the initial growth of invaded tumor (Morrone et al. 2006). Considering that NTPDase2 is the dominant E-NTPDase member expressed by astrocytes in culture (Wink et al. 2006) in a further study we better characterized the participation of nucleotides in glioma progression by restoring NTPDase2 expression and activity in rat C6 glioma cells. Surprisingly, NTPDase2 overexpression promoted a dramatic increase in the *in vivo* glioma growth and in the malignant characteristics (Fig. 5.2). A sizable platelet sequestration in the tumor area and an increase in angiogenesis and inflammatory response were observed (Braganhol et al. 2009). The opposite biological outcomes obtained by using NTPDase2 (high ATPase/ADPase ratio) and apyrase (low ATPase/ADPase ratio) as ATP/ADP scavengers, reveal a complex interactions between tumor, immune cells and purinergic mediators. This inverse effect could be due to the fact that whereas NTPDase1 hydrolyses ATP and ADP approximately equally well, the preferred degradation of ATP over ADP of NTPDase2 favors extracellular ADP accumulation (Robson et al. 2006; Zimmermann 2001). Considering that platelets express P2 receptors, which are activated by ADP ($P2Y_1$ and $P2Y_{12}$), we hypothesized that ADP produced by the NTPDase2 overexpressed in the implanted glioma cells could activate these receptors, leading to increased platelet recruitment and activation. The latter processes promote angiogenesis as well as the recruitment of other inflammatory cells (Sierko and Wojtukiewicz 2007). The treatment with clopidogrel, a $P2Y_{12}$ antagonist that prevents the platelet activation by ADP, decreased these parameters to control levels. These data suggest that the ADP derived from NTPDase2 activity stimulates platelet migration to the tumor area and that NTPDase2, by regulating angiogenesis and inflammation, seems to play an important role in tumor progression (Braganhol et al. 2009).

Interestingly, the NTPDase2 reconstitution in gliomas altered not only the local tumor development, but also modulated systemic inflammatory responses. NTPDase2 overexpression promoted an increase in IL-1 β , TNF- α and IL-6 pro-inflammatory cytokine production and regulated platelet func-

tion *in vivo*. Additionally, pathological alterations in the lungs were observed in rats bearing NTPDase2-gliomas (Braganhof et al. 2011). These data suggest that disruption of purinergic signaling creates an inflammatory microenvironment that dictates tumor cell progression and local invasiveness. Moreover, our findings reveal a previously underestimated role for ADP in tumor promotion and reinforce the important roles carried out by the different E-NTPDase members, which, by working in a highly coordinated enzymatic chain, maintain the extracellular nucleotide equilibrium and control the effects mediated by purinergic receptors.

As presented in the begging of this chapter, in concert with alterations in the extracellular ATP metabolism, modulations of P2 receptors, mainly P2Y₁, P2Y₂, P2Y₁₂ and P2X₇, may be important participants in glioma pathology. In C6 glioma cells P2Y₂ respond to ATP and UTP while P2Y₁ and P2Y₁₂ both respond to ADP. Baranska et al. have shown that agonists of these receptors modulate activities of ERK1/2 and PI3K, which are central pathways of cell survival (Baranska et al. 2004). These effects depend on physiological conditions of the cells. Under serum starvation culture conditions, UTP and ADP modulate positively ERK1/2 on C6 rat glioma. In non-starved cells, ADP markedly decreases the PI3K activity, whereas in serum-starved cells it causes an opposite effect. The differential P2Y expression under different culture conditions suggests a cross-talk between P2Y₁ and P2Y₁₂ receptors in order to favor the glioma cell growth (Baranska et al. 2004).

P2X₇ receptor subtype possesses unique biological properties such as the opening of a pore through which molecules up to 900 Da can pass. P2X₇ is responsible for ATP-induced cell death in various cell types through mechanisms that involve necrotic features such as swelling, loss of membrane integrity and apoptosis (Taylor et al. 2008; Tsukimoto et al. 2005). In the CNS, ATP is highly toxic to neurons *in vitro* and *ex vivo* (Morrone et al. 2005) and extracellular ATP plays an important role in neuronal death in pathological conditions such multiple sclerosis, Alzheimer and brain ischemia (Burnstock 2008). To date, the involvement and roles of P2X₇R in modulating the growth of brain tumors have been little explored. The P2X₇ activation in glioma cells mediates multiple effects that include cell death, survival and modulation of immune system. For example, in rat C6 cell line, C6 *ex vivo* glioma model and human U138MG, the involvement of P2X₇R in the resistance to cytotoxicity of ATP has been reported (Braganhof et al. 2008; Morrone et al. 2005). Consistent with the P2X₇R role in tumor progression, immunohistochemical staining of human glioblastoma tissue samples demonstrated greater expression of P2X₇R compared to control non-tumor samples and the pharmacological blockage of P2X₇R inhibits *in vivo* C6 glioma growth (Ryu et al. 2011). On the other hand, GL261 a murine glioma cell line sensitive to high concentrations of ATP, presents a higher ATPase activity, suggesting that the sensitivity may be compensated by a higher degradation rate of ATP for protective reasons. The sensitivity of GL261 to ATP and BzATP was blocked by silencing of P2X₇R (Tamajusuku et al. 2010). The cell death induced by ATP is mainly necrotic of nature, but their involvement in actual tumor growth is not clear. Blocking of P2X₇R with BBG, known P2X₇ receptor antagonist, produced a marked reduction in C6 glioma growth in rats, suggesting that cell death of glioma cells is not an important feature of this model (Ryu et al. 2010).

In conclusion, the information presented here supports the idea that alterations in the activity and expression of ectonucleotidases are involved in the glioma progression. The alterations in the extracellular ATP metabolism associated with P1/P2 receptor disruption may have important consequences in events related to tumor advance (Fig. 5.3). Although additional studies are needed to determine whether the altered nucleotide hydrolysis is the cause or consequence of malignant transformation, the ectonucleotidases may be considered as new molecular markers of gliomas and future target for pharmacological or gene therapy.

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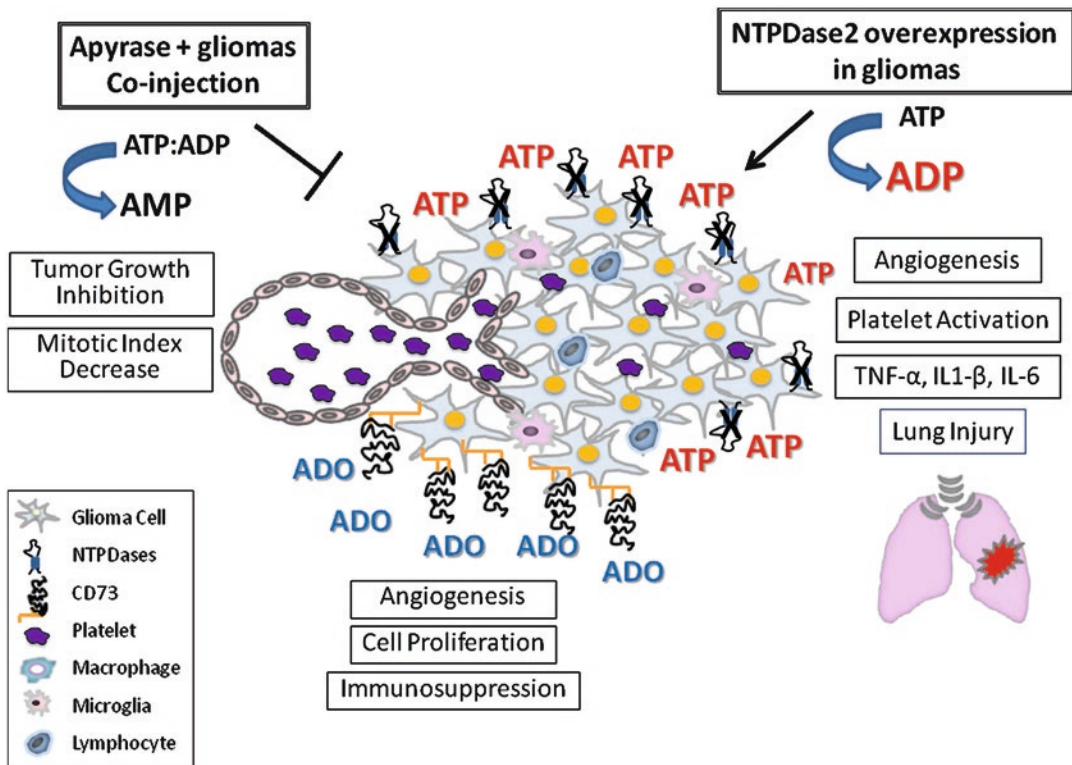


Fig. 5.3 Possible pathways connecting alterations in purinergic signaling and glioma progression. Gliomas exhibit an inversion in the extracellular nucleotide metabolism when compared to astrocytes in culture, hydrolyzing poorly the ATP and highly the AMP. This pattern of enzymatic activity may favor the accumulation of extracellular ATP and adenosine in the tumor milieu, inducing cell proliferation, angiogenesis and immunosuppression. Conversely, ATP could induce neuronal cell death, increasing the extracellular ATP pool and further improving the tumor invasion. In accordance with the participation of ATP and ADP in the glioma pathology, the co-injection of apyrase with gliomas decreased the tumor growth and the malignant characteristics, while NTPDase2 produced the opposite effect. We suggest that the ADP formation may be a component of platelet activation, induction of inflammatory cytokine release and the consequent tumor growth observed. The high expression of ecto-5'-NT/CD73, an adhesion and migration cell related protein and main source of enzymatic extracellular adenosine might mediate the interactions between tumor cells and tumor cell-ECM, essential for glioma progression

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References

- Agresti C, Meomartini ME, Amadio S, Ambrosini E, Serafini B, Franchini L, Volonte C, Aloisi F, Visentin S (2005) Metabotropic P2 receptor activation regulates oligodendrocyte progenitor migration and development. *Glia* 50:132–144
- Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444:756–760
- Baranska J, Czajkowski R, Sabala P (2004) Cross-talks between nucleotide receptor-induced signaling pathways in serum-deprived and non-starved glioma C6 cells. *Adv Enzyme Regul* 44:219–232

- Battastini AMO, da Rocha JBT, Kuplich CK, Dias RD, Sarkis JJF (1991) Characterization of an ATP diphosphohydrolase (EC 3.6.1.5) in synaptosomes from cerebral cortex of adult rats. *Neurochem Res* 16(12):1303–1310
- Bavaresco L, Bernardi A, Braganhol E, Wink MR, Battastini AMO (2007) Dexamethasone inhibits proliferation and stimulates ecto-5'-nucleotidase/CD73 activity in C6 rat glioma cell line. *J Neurooncol* 84:1–8
- Bavaresco L, Bernardi A, Braganhol E, Cappellari AR, Rockenbach L, Farias PF, Wink MR, Delgado-Cañedo A, Battastini AMO (2008) The role of ecto-5'-nucleotidase/CD73 in glioma cell line proliferation. *Mol Cell Biochem* 319(1–2):61–68
- Beier D, Rohrl S, Pillai DR, Schwarz S, Kunz-Schughart LA, Leukel P, Proescholdt M, Brawanski A, Bogdahn U, Trampe-Kieslich A, Giebel B, Wischhusen J, Reifenberger G, Hau P, Beier CP (2008) Temozolomide preferentially depletes cancer stem cells in glioblastoma. *Cancer Res* 68:5706–5715
- Belcher SM, Zsarnovszky A, Crawford PA, Hemani H, Spurling L, Kirley TL (2006) Immunolocalization of ectonucleoside triphosphate diphosphohydrolase 3 in rat brain: implications for modulation of multiple homeostatic systems including feeding and sleep-wake behavior. *Neuroscience* 137:1331–1346
- Beraudi A, Traversa U, Villani L, Sekino Y, Nagy JI, Poli A (2003) Distribution and expression of A₁ adenosine receptors, adenosine deaminase and adenosine deaminase-binding protein (CD26) in goldfish brain. *Neurochem Int* 42(6):455–464
- Berens ME, Giese A (1999) "...those left behind." Biology and oncology of invasive glioma cells. *Neoplasia* 1:208–219
- Bernardi A, Bavaresco L, Wink MR, Jacques-Silva MC, Delgado-Cañedo A, Lenz G, Battastini AMO (2007) Indomethacin stimulates activity and expression of ecto-5'-nucleotidase/CD73 in glioma cell lines. *Eur J Pharmacol* 569:8–15
- Bianchi V, Spychala J (2003) Mammalian 5'-nucleotidases. *J Biol Chem* 278(47):46195–46198
- Biederbick A, Kosan C, Kunz J, Elsassner HP (2000) First apyrase splice variants have different enzymatic properties. *J Biol Chem* 275:19018–19024
- Bigonnesse F, Lévesque SA, Kukulski F, Lecka J, Robson SC, Fernandes MJ, Sévigny J (2004) Cloning and characterization of mouse nucleoside triphosphate diphosphohydrolase-8. *Biochemistry* 43(18):5511–5519
- Blass-Kampmann S, Kindler-Rohrborn A, Deissler H, D'Urso D, Rajewsky MF (1997) In vitro differentiation of neural progenitor cells from prenatal rat brain: common cell surface glycoprotein on three glial cell subsets. *J Neurosci Res* 48:95–111
- Blay J, White TD, Hoskin DW (1997) The extracellular fluid of solid carcinomas contains immunosuppressive concentrations of adenosine. *Cancer Res* 57:2602–2605
- Boeck CR, Sarkis JJF, Vendite D (2002) Kinetic characterization and immunodetection of ecto-ATP diphosphohydrolase (EC 3.6.1.5) in cultured hippocampal neurons. *Neurochem Int* 40:449–453
- Bollen M, Gijssbers R, Ceulemans H, Stalmans W, Stefan C (2000) Nucleotide pyrophosphatases/phosphodiesterases on the move. *Crit Rev Biochem Mol Biol* 35:393–432
- Braganhol E, Tamajusuku ASK, Bernardi A, Wink MR, Battastini AMO (2007) Ecto-5'-nucleotidase/CD73 inhibition by quercetin in the human U138MG glioma cell line. *Biochim Biophys Acta* 1770:1352–1359
- Braganhol E, Huppés D, Bernardi A, Wink MR, Lenz G, Battastini AMO (2008) A comparative study of ectonucleotidase and P2 receptor mRNA profiles in C6 cell line cultures and C6 ex vivo glioma model. *Cell Tissue Res* 335(2):331–340
- Braganhol E, Morrone FB, Bernardi A, Huppés D, Meurer L, Edelweiss MI, Lenz G, Wink MR, Robson SC, Battastini AMO (2009) NTPDase2 expression modulates in vivo rat glioma growth. *Cancer Sci* 100(8):1434–1442
- Braganhol E, Zanin RF, Bernardi A, Bergamin LS, Cappellari AR, Campesato LF, Morrone FB, Campos MM, Calixto JB, Edelweiss MI, Wink MR, Sévigny J, Robson SC, Battastini AM (2011) Overexpression of NTPDase2 in gliomas promotes systemic inflammation and pulmonary injury. *Purinergic Signal*. doi:10.1007/s11302-011-9276-1
- Braun N, Sevigny J, Robson SC, Enjoji K, Guckelberger O, Hammer K, Di Virgilio F, Zimmermann H (2000) Assignment of ecto-nucleoside triphosphate diphosphohydrolase-1/CD39 expression to microglia and vasculature of the brain. *Eur J Neurosci* 12:4357–4366
- Braun N, Sevigny J, Mishra SK, Robson SC, Barth SW, Gerstberger R, Hammer K, Zimmermann H (2003) Expression of the ecto-ATPase NTPDase2 in the germinal zones of the developing and adult rat brain. *Eur J Neurosci* 17:1355–1364
- Bruno AN, Bonan CD, Wofchuk ST, Sarkis JJ, Battastini AM (2002) ATP diphosphohydrolase (NTPDase 1) in rat hippocampal slices and effect of glutamate on the enzyme activity in different phases of development. *Life Sci* 71(2):215–225
- Buckingham SC, Campbell SL, Haas BR, Montana V, Robel S, Ogunrinu T, Sontheimer H (2011) Glutamate release by primary brain tumors induces epileptic activity. *Nat Med* 17(10):1269–1274
- Burnstock G (2008) Purinergic signalling and disorders of the central nervous system. *Nat Rev Drug Discov* 7(7):575–590
- Calhau C, Martel F, Soares-da-Silva P, Hipólito-Reis C, Azevedo I (2002) Regulation of [(3)H]MPP(+) transport by phosphorylation/dephosphorylation pathways in RBE4 cells: role of ecto-alkaline phosphatase. *Naunyn-Schmiedeberg Arch Pharmacol* 365(5):349–356

- Cappellari AR, Vasques GJ, Bavaresco L, Braganhol E, Battastini AMO (2011) Involvement of ecto-5'-nucleotidase/CD73 in U138MG cell adhesion. *Mol Cell Biochem* 359:315–322
- Chadwick BP, Frischauf AM (1998) The CD39-like gene family: identification of three new human members (CD39L2, CD39L3, and CD39L4), their murine homologues, and a member of the gene family from *Drosophila melanogaster*. *Genomics* 50(3):357–367
- Ciruela F, Saura C, Canela EI, Mallol J, Lluís C, Franco R (1996) Adenosine deaminase affects ligand-induced signaling by interacting with cell surface adenosine receptors. *FEBS Lett* 380:219–223
- Cunha RA, Sebastião AM (1991) Extracellular metabolism of adenine nucleotides and adenosine in the innervated skeletal muscle of the frog. *Eur J Pharmacol* 197:83–92
- Cunha RA, Almeida T, Ribeiro JA (2000) Modification by arachidonic acid of extracellular adenosine metabolism and neuromodulatory action in the rat hippocampus. *J Biol Chem* 275(48):37572–37581
- Deissler H, Blass-Kampmann S, Bruyneel E, Mareel M, Rajewsky MF (1995a) Neural cell surface differentiation antigen gp130RB13-6 induces fibroblasts and glioma cells to express astroglial proteins and invasive properties. *FASEB J* 13:657–666
- Deissler H, Lottspeich F, Rajewsky MF (1995b) Affinity purification and cDNA cloning of rat neural differentiation and tumor cell surface antigen gp130RB13-6 reveals relationship to human and murine PC-1. *J Biol Chem* 270:9849–9855
- Delarasse C, Gonnord P, Galante M, Auger R, Daniel H, Motta I, Kanellopoulos JM (2009) Neural progenitor cell death is induced by extracellular ATP via ligation of P2X7 receptor. *J Neurochem* 109:846–857
- Demuth T, Berens ME (2004) Molecular mechanisms of glioma cell migration and invasion. *J Neurooncol* 70:217–228
- Dornand J, Bonnafous JC, Mani JC (1978) Purification and properties of 5'-nucleotidase from lymphocyte plasma membranes. *Eur J Biochem* 87:459–465
- Ehrlich YH, Hogan MV, Pawlowska Z, Naik U, Kornecki E (1990) Ectoprotein kinase in the regulation of cellular responsiveness to extracellular ATP. *Ann N Y Acad Sci* 603:401–416
- Fonta C, Negyessy L, Renaud L, Barone P (2004) Areal and subcellular localization of the ubiquitous alkaline phosphatase in the primate cerebral cortex: evidence for a role in neurotransmission. *Cereb Cortex* 14:595–609
- Fonta C, Negyessy L, Renaud L, Barone P (2005) Postnatal development of alkaline phosphatase activity correlates with the maturation of neurotransmission in the cerebral cortex. *J Comp Neurol* 486(2):179–196
- Franco R, Casado V, Ciruela F, Saura C, Mallol J, Canela EI, Lluís C (1997) Cell surface adenosine deaminase: much more than an ecto-enzyme. *Prog Neurobiol* 52:283–294
- Franco R, Valenzuela A, Lluís C, Blanco J (1998) Enzymatic and extraenzymatic role of ecto-adenosine deaminase in lymphocytes. *Immunol Rev* 161:27–42
- Fumagalli M, Brambilla R, D'Ambrosi N, Volonte C, Matteoli M, Verderio C, Abbracchio MP (2003) Nucleotide-mediated calcium signaling in rat cortical astrocytes: role of P2X and P2Y receptors. *Glia* 43:218–303
- Fuss B, Baba H, Phan T, Tuohy VK, Macklin WB (1997) Phosphodiesterase I, a novel adhesion molecule and/or cytokine involved in oligodendrocyte function. *J Neurosci* 17:9095–9103
- Ganzella MG, Faraco RB, Almeida RF, Fernandes VF, Souza DO (2011) Intracerebroventricular administration of inosine is anticonvulsant against quinolinic acid-induced seizures in mice: an effect independent of benzodiazepine and adenosine receptors. *Pharmacol Biochem Behav* 100:271–274
- Gessi S, Sacchetto V, Fogli E, Merighi S, Varani K, Baraldi PG, Tabrizi MA, Leung E, MacLennan S, Borea PA (2010) Modulation of metalloproteinase-9 in U87MG glioblastoma cells by A3 adenosine receptors. *Biochem Pharmacol* 79:1483–1495
- Gessi S, Merighi S, Sacchetto V, Simioni C, Borea PA (2011) Adenosine receptors and cancer. *Biochim Biophys Acta* 1808:1400–1412
- Gines S, Ciruela F, Burgueño J, Casado V, Canela EI, Mallol J, Lluís C, Franco R (2001) Involvement of caveolin in ligand-induced recruitment and internalization of A1 adenosine receptor and adenosine deaminase in an epithelial cell line. *Mol Pharmacol* 59:1314–1323
- Goding JW, Grobden B, Slegers H (2003) Physiological and pathophysiological functions of the ecto-nucleotide pyrophosphatase/phosphodiesterase family. *Biochim Biophys Acta* 1638:1–19
- Goldman N, Chen M, Fujita T, Xu Q, Peng W, Liu W, Jensen TK, Pei Y, Wang F, Han X, Chen JF, Schnermann J, Takano T, Bekar L, Tieu K, Nedergaard M (2010) *Nat Neurosci* 13(7):883–888
- Grobden B, Anciaux K, Roymans D, Stefan C, Bollen M, Esmans EL, Slegers H (1999) An ecto-nucleotide pyrophosphatase is one of the main enzymes involved in the extracellular metabolism of ATP in rat C6 glioma. *J Neurochem* 72:826–834
- Hansen KR, Resta R, Webb CF, Thompson LF (1995) Isolation and characterization of the promoter of the human 5'-nucleotidase 9CD730-encoding gene. *Gene* 167:307–312
- Haskó G, Sitkovsky MV, Szabó C (2004) Immunomodulatory and neuroprotective effects of inosine. *Trends Pharmacol Sci* 25(3):152–157

- Heine P, Braun N, Heilbronn A, Zimmermann H (1999) Functional characterization of rat ecto-ATPase and ecto-ATP diphosphohydrolase after heterologous expression in CHO cells. *Eur J Biochem* 262:102–107
- Heymann D, Reddington M, Kreutzberg GW (1984) Subcellular localization of 5'-nucleotidase in rat brain. *J Neurochem* 43(4):971–978
- Hicks-Berger CA, Chadwick BP, Frischauf AM, Kirley TL (2000) Expression and characterization of soluble and membrane-bound human nucleoside triphosphate diphosphohydrolase 6 (CD39L2). *J Biol Chem* 275:34041–34045
- Jacques-Silva MC, Bernardi A, Rodnight R, Lenz G (2004a) ERK, PKC and PI3K/Akt pathways mediate extracellular ATP and adenosine-induced proliferation of U138-MG human glioma cell line. *Oncology* 67:450–459
- Jacques-Silva MC, Rodnight R, Lenz G, Liao Z, Kong Q, Tran M, Kang Y, Gonzalez FA, Weisman GA, Neary JT (2004b) P2X7 receptors stimulate AKT phosphorylation in astrocytes. *Br J Pharmacol* 141:1106–1117
- Joseph SM, Pifer MA, Przybylski RJ, Dubyak GR (2004) Methylene ATP analogs as modulators of extracellular ATP metabolism and accumulation. *Br J Pharmacol* 142:1002–1014
- Kaczmarek E, Koziak K, Sevigny J, Siegel JB, Anrather J, Beaudoin AR, Bach FH, Robson SC (1996) Identification and characterization of CD39/vascular ATP diphosphohydrolase. *J Biol Chem* 271:33116–33122
- Kegel B, Braun N, Heine P, Maliszewski CR, Zimmermann H (1997) An ecto-ATPase and an ecto-ATP diphosphohydrolase are expressed in rat brain. *Neuropharmacology* 36(9):1189–1200
- Kiss DS, Zsarnovszky A, Horvath K, Gyorffy A, Bartha T, Hazai D, Sotonyi P, Somogyi V, Frenyo LV, Diano S (2009) Ecto-nucleoside triphosphate diphosphohydrolase 3 in the ventral and lateral hypothalamic area of female rats: morphological characterization and functional implications. *Reprod Biol Endocrinol* 7:31–43
- Knowles AF (2011) The GDA1_CD39 superfamily: NTPDases with diverse functions. *Purinergic Signal* 7:21–45
- Komoszynski M, Wojtczak A (1996) Apyrases (ATP diphosphohydrolases, EC 3.6.1.5): function and relationship to ATPases. *Biochim Biophys Acta* 1310:233–241
- Koshiha M, Kojima H, Huang S, Apasov S, Sitkovsky MV (1997) Memory of extracellular adenosine A2A purinergic receptor-mediated signalling in murine T cells. *J Biol Chem* 272:25881–25889
- Kubler D, Pyerin W, Bill O, Hotz A, Sonka J, Kinzel V (1989) Evidence for ecto-protein kinase activity that phosphorylates kemptide in a cyclic AMP-dependent model. *J Biol Chem* 264(24):14549–14555
- Ledur PF, Villodre ES, Paulus R, Cruz LA, Flores DG, Lenz G (2011) Extracellular ATP reduces tumorsphere growth and cancer stem cell population in U87 human glioblastoma cells. *J Cell Biochem*. doi:10.1002/s11302-011-9252-9
- Lemoli RM, Ferrari D, Fogli M, Rossi L, Pizzirani C, Forchap S, Chiozzi P, Vaselli D, Bertoli F, Foutz T, Aluigi M, Baccarani M, Di Virgilio F (2004) Extracellular nucleotides are potent stimulators of human hematopoietic stem cells in vitro and in vivo. *Blood* 104:1662–1670
- Lenz G, Gottfried C, Luo Z, Avruch J, Rodnight R, Nie WJ, Kang Y, Neary JT (2000) P2Y purinergic receptor subunits recruit different Mek activators in astrocytes. *Br J Pharmacol* 129:927–936
- Lenz G, Goncalves D, Luo Z, Avruch J, Rodnight R, Neary JT (2001) Extracellular ATP stimulates an inhibitory pathway towards growth factor-induced cRaf-1 and MEKK activation in astrocyte cultures. *J Neurochem* 77:1001–1009
- Lie AA, Blümcke I, Beck H, Wiestler OD, Elger CE, Schoen SW (1999) 5'-Nucleotidase activity indicates sites of synaptic plasticity and reactive synaptogenesis in the human brain. *J Neuropathol Exp Neurol* 58(5):451–458
- Liu C, Sage JC, Miller MR, Verhaak RG, Hippenmeyer S, Vogel H, Foreman O, Bronson RT, Nishiyama A, Luo L, Zong H (2011) Mosaic analysis with double markers reveals tumor cell of origin in glioma. *Cell* 146:209–221
- Lohmann K (1928) The isolation of various natural phosphoric acid compounds and their identity. *Biochem Z* 194:306–327
- Louis DN (1994) The p53 gene and protein in human brain tumors. *J Neuropathol Exp Neurol* 53:11–21
- Ludwig HC, Rausch S, Schallock K, Markakis E (1999) Expression of CD73 (ecto-5'-nucleotidase) in 165 glioblastomas by immunohistochemistry and electromicroscopic histochemistry. *Anticancer Res* 19:1747–1752
- Maeda T, Nishiyama F, Ogashiwa M, Takeuchi K, Hirano H (1985) Phosphatase activities in human glioma cells as revealed by light and electron microscopy—a preliminary study. *J Neurooncol* 3(3):211–216
- Maher EA, Furnari FB, Bachoo RM, Rowitch DH, Louis DN, Cavenee WK, DePinho RA (2001) Malignant glioma: genetics and biology of a grave matter. *Genes Dev* 15:1311–1333
- Marcus HJ, Carpenter KL, Price SJ, Hutchinson PJ (2010) In vivo assessment of high-grade glioma biochemistry using microdialysis: a study of energy-related molecules, growth factors and cytokines. *J Neurooncol* 97:11–23
- Martins MJ, Negrão MR, Hipólito-Reis C (2001) Alkaline phosphatase from rat liver and kidney is differentially modulated. *Clin Biochem* 34:463–468
- Matsuoka I, Ohkubo S (2004) ATP- and adenosine-mediated signaling in the central nervous system: adenosine receptor activation by ATP through rapid and localized generation of adenosine by ecto-nucleotidases. *J Pharmacol Sci* 94:95–99
- Matute C (2008) P2X7 receptors in oligodendrocytes: a novel target for neuroprotection. *Mol Neurobiol* 38:123–128

- Melani A, De Micheli E, Pinna G, Alfieri A, Corte LD, Pedata F (2003) Adenosine extracellular levels in human brain gliomas: an intraoperative microdialysis study. *Neurosci Lett* 346(1–2):93–96
- Merighi S, Mirandola P, Varani K, Gessi S, Leung E, Baraldi PG, Tabrizi MA, Borea PA (2003) A glance at adenosine receptors: novel target for antitumor therapy. *Pharmacol Ther* 100:31–48
- Meyerhof O (1945) The origin of reaction of Harden and Young in cell-free alcoholic fermentation. *J Biol Chem* 157:105–109
- Morrone FB, Jacques-Silva MC, Horn AP, Bernardi A, Schwartzmann G, Rodnight R, Lenz G (2003) Extracellular nucleotides and nucleosides induce proliferation and increase nucleoside transport in human glioma cell line. *J Neurooncol* 64:211–218
- Morrone FB, Horn AP, Stella J, Spiller F, Sarkis JJ, Salbego CG, Lenz G, Battastini AM (2005) Increased resistance of glioma cell lines to extracellular ATP cytotoxicity. *J Neurooncol* 71:135–140
- Morrone FB, Oliveira DL, Gamermann P, Stella J, Wofchuk S, Wink MR, Meurer L, Edelweiss MIA, Lenz G, Battastini AMO (2006) In vivo glioblastoma growth is reduced by apyrase activity in a rat glioma model. *BMC Cancer* 23:226
- Mulero JJ, Yeung G, Nelken ST, Ford JE (1999) CD39-L4 is a secreted human apyrase, specific for the hydrolysis of nucleoside diphosphates. *J Biol Chem* 274:20064–20067
- Mulero JJ, Yeung G, Nelken ST, Bright JM, McGowan DW, Ford JE (2000) Biochemical characterization of CD39L4. *Biochemistry* 39(42):12924–12928
- Murphy-Piedmonte DM, Crawford PA, Kirley TL (2005) Bacterial expression, folding, purification and characterization of soluble NTPDase5 (CD39L4) ecto-nucleotidase. *Biochim Biophys Acta* 1747:251–259
- Nagy AK, Shuster TA, Delgado-Escueta AV (1986) Ecto-ATPase of mammalian synaptosomes: identification and enzymic characterization. *J Neurochem* 47:976–986
- Nagy A, Shuster TA, Delgado-Escueta AV (1989) Rat brain synaptosomal ATP:AMP-phosphotransferase activity. *J Neurochem* 53:1166–1172
- Narisawa S, Hasegawa H, Watanabe K, Millán JL (1994) Stage-specific expression of alkaline phosphatase during neural development of the mouse. *Dev Dyn* 201:227–235
- Navarro JM, Olmo N, Turnay J, López-Conejo MT, Lizarbe MA (1998) Ecto-5'-nucleotidase from a human colon adenocarcinoma cell line. Correlation between enzyme activity and levels in intact cells. *Mol Cell Biochem* 187:121–131
- Neary JT, Kang Y, Willoughby KA, Ellis EF (2003) Activation of extracellular signal-regulated kinase by stretch-induced injury in astrocytes involves extracellular ATP and P2 purinergic receptors. *J Neurosci* 23:2348–2356
- Ohkubo S, Kimura J, Matsuoka I (2000) Ecto-alkaline phosphatase in NG108-15 cells: a key enzyme mediating P1 antagonist-sensitive ATP response. *Br J Pharmacol* 131(8):1667–1672
- Oses JP, Cardoso CM, Germano RA, Kirst IB, Rücker B, Fürstenau CR, Wink MR, Bonan CD, Battastini AM, Sarkis JJ (2004) Soluble NTPDase: an additional system of nucleotide hydrolysis in rat blood serum. *Life Sci* 74(26):3275–3284
- Paas Y, Bohana-Kashtan O, Fishelson Z (1999) Phosphorylation of the complement component, C9, by an ecto-protein kinase of human leukemic cells. *Immunopharmacology* 42:175–185
- Paez JG, Recio JA, Rouzaut A, Notario V (2001) Identity between the PCPH proto-oncogene and the CD39L4 (NTPDase5) ectonucleoside triphosphate diphosphohydrolase gene. *Int J Oncol* 19:1249–1254
- Picher M, Burch LH, Hirsh AJ, Spychala J, Boucher RC (2003) Ecto 5'-nucleotidase and nonspecific alkaline phosphatase. Two AMP-hydrolyzing ecto-enzymes with distinct roles in human airways. *J Biol Chem* 278:13468–13479
- Pinsky DJ, Broekman MJ, Peschon JJ, Stocking KL, Fujita T, Ramasamy R, Connolly ES Jr, Huang J, Kiss S, Zhang Y, Choudhri TF, McTaggart RA, Liao H, Drosopoulos JH, Price VL, Marcus AJ, Maliszewski CR (2002) Elucidation of the thromboregulatory role of CD39/ectoapyrase in the ischemic brain. *J Clin Invest* 109:1031–1040
- Plesner L (1995) Ecto-ATPases: identities and functions. *Int Rev Cytol* 158:141–214
- Ravera S, Aluigi MG, Calzia D, Ramoino P, Morelli A, Panfoli I (2011) Evidence for ectopic aerobic ATP production on C6 glioma cell plasma membrane. *Cell Mol Neurobiol* 31:313–321
- Recio JA, Paez JG, Maskeri B, Loveland M, Velasco JA, Notario V (2000) Both normal and transforming PCPH proteins have guanosine diphosphatase activity but only the oncoprotein cooperates with Ras in activating extracellular signal-regulated kinase ERK1. *Cancer Res* 60:1720–1728
- Recio JA, Paez JG, Sanders S, Kawakami T, Notario V (2002) Partial depletion of intracellular ATP mediates the stress-survival function of the PCPH oncoprotein. *Cancer Res* 62:2690–2694
- Redegeld FA, Caldwell CC, Sitkovsky MV (1999) Ecto-protein kinases: ecto-domain phosphorylation as a novel target for pharmacological manipulation? *Trends Pharmacol Sci* 20:453–459
- Resta R, Yamashita Y, Thompson LF (1998) Ecto-enzyme and signaling functions of lymphocyte. *Immunol Rev* 161:95–109
- Ricci-Vitiani L, Pallini R, Biffoni M, Todaro M, Invernici G, Cenci T, Maira G, Parati EA, Stassi G, Larocca LM, De Maria R (2010) Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature* 468:824–828

- Robson SC, Sévigny J, Zimmermann H (2006) The E-NTPDase family of ectonucleotidases: structure function relationship and pathophysiological significance. *Purinergic Signal* 2:409–430
- Rouzaut A, Recio JA, Notario V (2001) Expression of the protein product of the PCHP proto-oncogene in human tumor cell lines. *Radiat Res* 155:181–187
- Ryu JK, Choi HB, Hatori K, Heisel RL, Pelech SL, McLarnon JG, Kim SU (2003) Adenosine triphosphate induces proliferation of human neural stem cells: role of calcium and p70 ribosomal protein S6 kinase. *J Neurosci Res* 72:352–362
- Ryu JK, Jantaratnotai N, Serrano-Perez MC, McGeer PL, McLarnon JG (2010) Block of purinergic P2X7R inhibits tumor growth in a C6 glioma brain tumor animal model. *J Neuropathol Exp Neurol* 70:13–22
- Ryu JK, Jantaratnotai N, Serrano-Perez MC, McGeer PL, McLarnon JG (2011) Block of purinergic P2X7R inhibits tumor growth in a C6 glioma brain tumor animal model. *J Neuropathol Exp Neurol* 70(1):13–22
- Sadej R, Spychala J, Skladanowski AC (2006) Expression of ecto-5'-nucleotidase (eN, CD73) in cell lines from various stages of human melanoma. *Melanoma Res* 16(3):213–222
- Saura C, Mallol J, Canela E, Lluis C, Franco R (1998) Adenosine deaminase and A1 adenosine receptors internalize together following agonist-induced receptor desensitization. *J Biol Chem* 273:17610–17617
- Seehafer J, Longenecker BM, Shaw AR (1984) Biochemical characterization of human carcinoma surface antigen associated with protein kinase activity. *Int J Cancer* 34(6):821–829
- Shapiro JR (2001) Genetics of brain neoplasms. *Curr Neurol Neurosci Rep* 1:217–224
- Shi JD, Kukar T, Wang CY, Li QZ, Cruz PE, Davoodi-Semiromi A, Yang P, Gu Y, Lian W, Wu DH, She JX (2001) Molecular cloning and characterization of a novel mammalian endo-apyrase (LALP1). *J Biol Chem* 276:17474–17478
- Shukla V, Zimmermann H, Wanf L, Kettenmann H, Raab S, Hammer K, Sévigny J, Robson SC, Braun N (2005) Functional expression of the ecto-ATPase NTPDase2 and of nucleotide receptors by neuronal progenitor cells in the adult murine hippocampus. *Neurosci Res* 80(5):600–610
- Sierko E, Wojtukiewicz MZ (2007) Inhibition of platelet function: does it offer a chance of better cancer progression control? *Semin Thromb Hemost* 33:712–721
- Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB (2003) Identification of a cancer stem cell in human brain tumors. *Cancer Res* 63:5821–5828
- Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB (2004) Identification of human brain tumour initiating cells. *Nature* 432:396–401
- Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT Jr, Carter BS, Krichevsky AM, Breakefield XO (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 10:1470–1476
- Smith TM, Carl SA, Kirley TL (1998) Immunological detection of ecto-ATPase in chicken and rat tissues: characterization, distribution, and a cautionary note. *Biochem Mol Biol Int* 45(5):1057–1066
- Spychala J (2000) Tumor-promoting functions of adenosine. *Pharmacol Ther* 87(2–3):161–173
- Spychala J, Zimmermann AG, Mitchell BS (1999) Tissue-specific regulation of the Ecto-5'-nucleotidase promoter. *J Biol Chem* 274:22705–22712
- Stanojević I, Bjelobaba I, Nedeljković N, Drakulić D, Petrović S, Stojiljković M, Horvat A (2011) Ontogenetic profile of ecto-5'-nucleotidase in rat brain synaptic plasma membranes. *Int J Dev Neurosci* 29(4):397–403
- Stefan C, Jansen S, Bollen M (2006) Modulation of purinergic signaling by NPP-type ectophosphodiesterases. *Purinergic Signal* 2:361–370
- Stochaj U, Dieckhoff J, Mollenhauer J, Cramer M, Mannherz HG (1989) Evidence for the direct interaction of chicken gizzard 5'-nucleotidase with laminin and fibronectin. *Biochim Biophys Acta* 992(3):385–392
- Stracke ML, Krutzsch HC, Unsworth EJ, Arestad A, Cioce V, Schiffmann E, Liotta LA (1992) Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulation protein. *J Biol Chem* 267:2524–2529
- Su AI, Welsh JB, Sapinoso LM, Kern SG, Dimitrov P, Lapp H, Schultz PG, Powell SM, Moskaluk CA, Frierson HF Jr, Hampton GM (2001) Molecular classification of human carcinomas by use of gene expression signatures. *Cancer Res* 61(20):7388–7393
- Takahashi T, Old LJ, Boyse EA (1970) Surface alloantigens of plasma cells. *J Exp Med* 131:1325–1341
- Takano T, Lin JH, Arcuino G, Gao K, Yang J, Nedergaard M (2001) Glutamate release promotes growth of malignant gliomas. *Nat Med* 7:1010–1015
- Tamajusuku AS, Villodre ES, Paulus R, Coutinho-Silva R, Battasstini AM, Wink MR, Lenz G (2010) Characterization of ATP-induced cell death in the GL261 mouse glioma. *J Cell Biochem* 109:983–991
- Taylor SR, Gonzalez-Begne M, Dewhurst S, Chimini G, Higgins CF, Melvin JE, Elliott JI (2008) Sequential shrinkage and swelling underlie P2X7-stimulated lymphocyte phosphatidylserine exposure and death. *J Immunol* 180:300–308
- TCGA (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455:1061–1068

- Tombetta ES, Helenius Q (1999) Glycoprotein reglucosylation and nucleotide sugar utilization in the secretory pathway: identification of a nucleoside diphosphatase in the endoplasmic reticulum. *EMBO J* 18:3282–3292
- Tsukimoto M, Harada H, Ikari A, Takagi K (2005) Involvement of chloride in apoptotic cell death induced by activation of ATP-sensitive P2X7 purinoceptor. *J Biol Chem* 280:2653–2658
- Turnay J, Olmo N, Reence G, Von der Mark K, Lizarbe MA (1989) 5'-Nucleotidase activity in cultured cell lines: effect of different assay conditions and correlation with cell proliferation. *In Vitro Cell Dev Biol* 25:1055–1061
- Velasco JA, Avila MA, Notario V (1999) The product of the cph oncogene is a truncated, nucleotide-binding protein that enhances cellular survival to stress. *Oncogene* 18:689–701
- Verkhatsky A, Krishtal OA, Burnstock G (2009) Purinoceptors on neuroglia. *Mol Neurobiol* 39:190–208
- Villar J, Arenas MI, MacCarthy CM, Blaquez MJ, Tirado OM, Notario V (2007) PCPH/ENTPD5 expression enhances the invasiveness of human prostate cancer cells by a protein kinase CD-dependent mechanism. *Cancer Res* 67(22):10859–10868
- Visvader JE (2011) Cells of origin in cancer. *Nature* 469:314–322
- Vlashi E, Kim K, Lagadec C, Donna LD, McDonald JT, Eghbali M, Sayre JW, Stefani E, McBride W, Pajonk F (2009) In vivo imaging, tracking, and targeting of cancer stem cells. *J Natl Cancer Inst* 101:350–359
- Vogel M, Kowalewski HJ, Zimmermann H, Janetzko A, Margolis RU, Wollny HE (1991) Association of the HNK-1 epitope with 5'-nucleotidase from *Torpedo marmorata*. *Biochem J* 278:199–202
- Vollmayer P, Koch M, Braun N, Heine P, Servos J, Israr E, Kegel B, Zimmermann H (2001) Multiple ecto-nucleotidase in PC12 cells: identification and cellular distribution after heterologous expression. *J Neurochem* 78:1019–1028
- Von Deimling A, Louis DN, Wiestler OD (1995) Molecular pathways in the formation of gliomas. *Glia* 15:328–338
- Wang T-F, Guidotti G (1999) Widespread expression of ecto-apyrase (CD39) in the central nervous system. *Brain Res* 790:318–322
- Wang R, Chadalavada K, Wilshire J, Kowalik U, Hovinga KE, Geber A, Fligelman B, Leversha M, Brennan C, Tabar V (2010) Glioblastoma stem-like cells give rise to tumour endothelium. *Nature* 468:829–833
- White N, Burnstock G (2006) P2 receptors and cancer. *Trends Pharmacol Sci* 27:211–217
- Wink MR, Lenz G, Braganhol E, Tamajusuku AS, Schwartzmann G, Sarkis JJ, Battastini AM (2003) Altered extracellular ATP, ADP and AMP catabolism in glioma cell lines. *Cancer Lett* 198(2):211–218
- Wink MR, Braganhol E, Tamajusuku ASK, Lenz G, Zerbini LF, Libermann TA, Sévigny J, Battastini AM, Robson SC (2006) Nucleoside triphosphate diphosphohydrolase-(NTPDase2/CD39L1) is the dominant ectonucleotidase expressed by rat astrocytes. *Neuroscience* 138:421–432
- Yamamoto T, Geiger JD, Daddona PE, Nagy JI (1987) Subcellular, regional and immunohistochemical localization of adenosine deaminase in various species. *Brain Res Bull* 19:473–484
- Ye ZC, Sontheimer H (1999) Glioma cells release excitotoxic concentrations of glutamate. *Cancer Res* 59:4383–4391
- Yegutkin GG (2008) Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signaling cascade. *Biochim Biophys Acta* 1783(5):673–694
- Yegutkin GG, Henttinen T, Samburski SS, Spychala J, Jalkanen S (2002) The evidence for two opposite, ATP-generating and ATP-consuming, extracellular pathways on endothelial and lymphoid cells. *Biochem J* 367:121–128
- Yeung G, Mulero JJ, McGowan DW, Bajwa SS, Ford JE (2000) CD39L2, a gene encoding a human nucleoside diphosphatase, predominantly expressed in the heart. *Biochemistry* 39(42):12916–12923
- Zhi X, Chen S, Zhou P, Shao Z, Wang L, Ou Z, Yin L (2007) RNA interference of ecto-5'-nucleotidase (CD73) inhibits human breast cancer cell growth and invasion. *Clin Exp Metastasis* 24(6):439–448
- Zhou P, Zhi X, Zhou T, Chen S, Li X, Wang L, Yin L, Shao Z, Ou Z (2007) Overexpression of Ecto-5'-nucleotidase (CD73) promotes T-47D human breast cancer cells invasion and adhesion to extracellular matrix. *Cancer Biol Ther* 6(3):426–431
- Zimmermann H (1992) 5'-Nucleotidase: molecular structure and functional aspects. *Biochem J* 285:345–365
- Zimmermann H (1996) Biochemistry, localization and functional roles of ecto-5'-nucleotidase in the nervous system. *Prog Neurobiol* 49(6):589–618
- Zimmermann H (2001) Ectonucleotidases: some recent developments and a note on nomenclature. *Drug Dev Res* 52:44–56
- Zimmermann H (2006) Purinergic signalling in neuron-glia interactions. In: *Novartis foundation symposium* 276, Wiley, Chichester, p 113–130
- Zimmermann H (2006) Ectonucleotidases in the nervous system. *Novartis Found Symp* 276:113–128
- Zimmermann H, Braun N, Kegel B, Heine P (1998) New insights into molecular structure and function of ecto-nucleotidases in the nervous system. *Neurochem Res* 32:421–425