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Review

Modulation of purinergic signaling by NPP-type ectophosphodiesterases

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Abstract

Extracellular nucleotides can elicit a wide array of cellular responses by binding to specific purinergic receptors. The level of ectonucleotides is dynamically controlled by their release from cells, synthesis by ectonucleoside diphosphokinases and ectoadenylate kinases, and hydrolysis by ectonucleotidases. One of the four structurally unrelated families of ectonucleotidases is represented by the NPP-type ectophosphodiesterases. Three of the seven members of the NPP family, namely NPP1–3, are known to hydrolyze nucleotides. The enzymatic action of NPP1–3 (in)directly results in the termination of nucleotide signaling, the salvage of nucleotides and/or the generation of new messengers like ADP, adenosine or pyrophosphate. NPP2 is unique in that it hydrolyzes both nucleotides and lysophospholipids and, thereby, generates products that could synergistically promote cell motility. We review here the enzymatic properties of NPPs and analyze current evidence that links their nucleotide-hydrolyzing capability to epithelial and neural functions, the immune response and cell motility.

Abbreviations: Ado – adenosine; ADP – adenosine 5′ diphosphate; AMP – adenosine 5′ monophosphate; Ap_nA – diadenosine polyphosphate; ATP – adenosine 5′ triphosphate; CSF – cerebrospinal fluid; E-NTPDases – ectonucleoside triphosphate diphosphohydrolases; GPC – glycerophosphorylcholine; LPA – lysophosphatidic acid; LPC – lysophosphatidyl choline; MCC – mucociliary clearing; NAD^+ – nicotinamide adenine dinucleotide; NMN – nicotinamide mononucleotide; NPP – nucleotide pyrophosphatase/phosphodiesterase; P_i – inorganic phosphate; PP_i – pyrophosphate; SM – sphingomyelin; SMB – somatomedin-B like domain; S-S bridges – disulfide bridges; UDP-glucose – uridine diphosphate glucose

Introduction

Extracellular nucleotides function as autocrine or paracrine signaling molecules. Their binding to P2 purinergic receptors leads to both short-term and long-term effects [1]. Short-term effects, like neurotransmission and hormone secretion, are mediated by the ionotropic P2X receptors that bind mainly ATP, while long-term effects, including cell proliferation, differentiation and migration, occur via the metabotropic G-protein coupled P2Y receptors that interact with a broader range of nucleotides [2–5]. In keeping with their importance in cell signaling, the extracellular concentration of nucleotides is tightly regulated. Nucleotides can be released from cells primarily by exocytosis or selective transport through the plasma membrane [1, 6], but they can also be generated extracellularly by nucleoside diphosphokinase and adenylate kinase activities [7–9] (Figure 1). There are four major families of ectonucleotidases, namely E-NTPDases (ectonucleoside triphosphate diphosphohydrolases), alkaline phosphatases, NPP-type ectophosphodiesterases and the 5′-nucleotidase [10]. Often these ectonucleotidases work in concert or consecutively (Figure 1). ATP, for instance, can be degraded in one step to either ADP or AMP by E-NTPDase or NPP isoenzymes. ADP can be further hydrolyzed to AMP by E-NTPDases and NPPs, and AMP is converted to adenosine by alkaline phosphatases or the 5′-nucleotidase. This sequential degradation mechanism not only terminates ATP signaling but also generates intermediates with distinct signaling properties. Thus, ADP selectively interacts with P2 receptor subtypes and is the principal platelet recruiting factor [11, 12], while adenosine, acting through P1 receptors, plays a major role in the regulation of blood flow and the immune response [13–15]. The relative contribution of the distinct ectonucleotidase species to the modulation of purinergic signaling may depend on differential tissue and cell distribution, regulation of expression, targeting to specific membrane domains, but also on substrate availability and substrate preference. For example, the localization of NTPDase1 and

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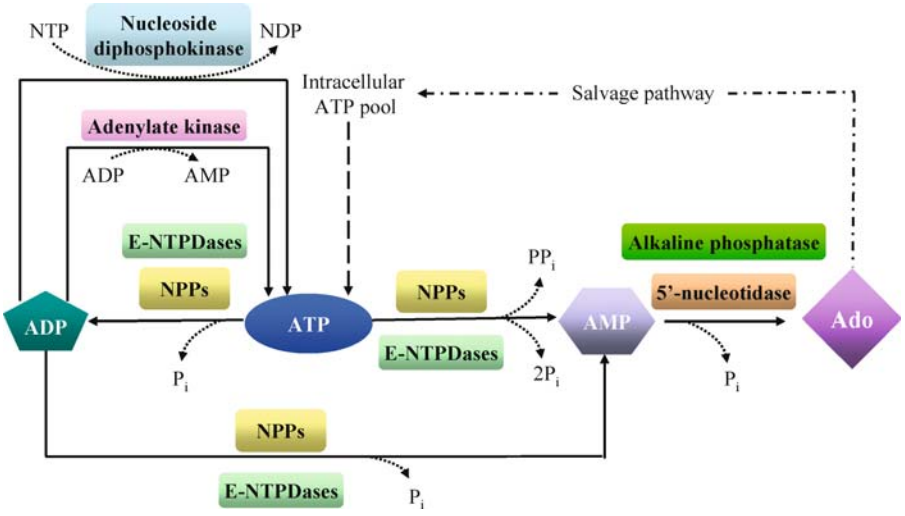

Figure 1. NPPs are part of the extracellular nucleotide-metabolizing network. The concentration of nucleotides in the extracellular milieu is the net result of the release of nucleotides from cells, their synthesis by nucleoside diphosphokinases and adenylate kinases, and their hydrolysis by ectonucleotidases. The examples shown here apply to ATP, the prototype extracellular nucleotide. Members of the E-NTDPase family, also known as apyrases, generally act as ATP diphosphohydrolases and hydrolyze ATP to ADP + P_i, and ADP to AMP + P_i, or ATP directly to AMP + 2P_i. Individual members however display substrate preference. For instance, E-NTPDase-1 metabolizes equally well ATP and ADP, while E-NTPDase-2 prefers ATP as a substrate. ATP can be regenerated from ADP by nucleoside diphosphokinase or adenylate kinase. NPPs, at least NPP1-3, have a nucleotide pyrophosphatase activity and metabolize ATP directly to ADP + P_i or to AMP + PP_i. The hydrolysis of AMP to adenosine by 5′-nucleotidase/CD73 completes the dephosphorylation pathway of ATP. Adenosine can be taken up by cells such as lymphocytes and be re-used for intracellular nucleotide synthesis (nucleotide salvage). Several ectonucleotide species can be expressed by a given cell type, but the relative abundance, sorting to specific membrane domains and substrate availability are ultimately responsible for the net outcome of the nucleotide metabolism at the cell surface.

NTPDase2 to distinct cell types within the vascular wall, combined with their substrate preference, may have direct implications for the control of platelet activation and coagulation responses *in vivo* [16, 17]. The former ectonucleotidase metabolizes both ATP and ADP while the latter acts primarily as an ATP phosphohydrolase.

The family of NPPs (nucleotide pyrophosphatases/phosphodiesterases) consists of seven members, numbered according to their order of cloning (Figure 2). Only NPP1-3, which have a common ancestor, have been implicated in the hydrolysis of nucleotides [10, 18, 19], while NPP6–7 are only known to hydrolyze phosphodiester bonds in lysophospholipids or other choline phosphodiesters [20–22]. Remarkably, NPP2 acts on both nucleotides and lysophospholipids [23, 24]. Established nucleotide(-derived) substrates of NPP1–3 include ATP, diadenosine polyphosphates (Ap_nA), UDP-glucose, NAD^+ and 3′-phosphoadenosine-5′-phosphosulfate (Figure 2). In vitro, NPP isoenzymes have an alkaline optimum pH, but the physiological relevance of this property has not been explored.

Except for NPP2, all NPPs are single-span transmembrane proteins. NPP1 and NPP3 have a type-II orientation, with their N-terminus facing the cytosol, while NPP4–7

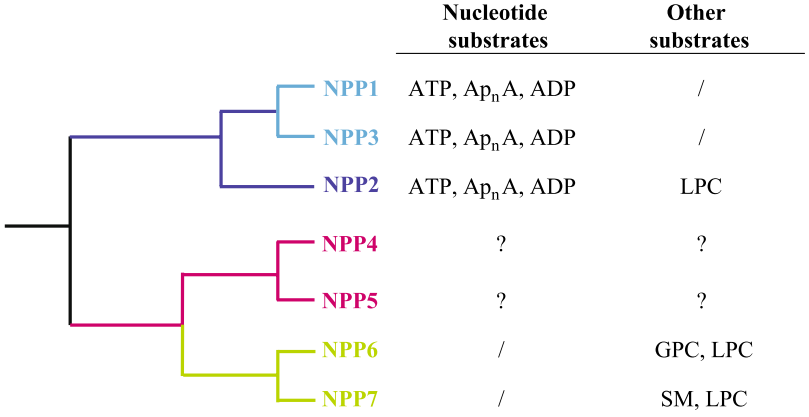

Figure 2. The phylogenetic tree of the NPP-family and some key substrates. Protein sequences for the human isoforms were retrieved from Genbank and aligned with CLUSTAL W. Accession numbers are: hNPP1, P22413; hNPP2, NP_006200.2; hNPP3, NP_005012.1; hNPP4, AAH18054.1; hNPP5, CAB56566.1; hNPP6, NP_699174.1; hNPP7, AAH41453.2. Representative nucleotide and/or lipid substrates are shown for each NPP isozyme. NPP1-3 have a common ancestor, and are the only known NPPs capable of hydrolyzing nucleotides. The overall aminoacid identity for the human isoenzymes, as obtained by Blast-2 sequence analysis at the NCBI site, is 41% (NPP1–NPP2), 52% (NPP1–NPP3) and 40% (NPP2–NPP3). *LPC* Lysophosphatidylcholine, *GPC* glycerophosporylcholine, *SM* sphingomyelin.

have been predicted to adopt a type-I orientation, with their N-terminus facing the extracellular milieu [18, 20–22] (Figure 3). This prediction is supported experimentally for NPP6 and NPP7. Thus, truncated forms of NPP6 or NPP7 lacking the C-terminal putative transmembrane domain are not retained at the plasma membrane and are secreted [22, 25]. In addition, a soluble form of NPP7 is released from the plasma membrane by trypsin, which acts on tryptic arginine site(s) located upstream of the transmembrane domain. Soluble forms have also been identified for NPP1, NPP3 and NPP6, but the mechanisms for their generation remains to be elucidated [22, 26–29]. NPP2 is synthesized as a pre-pro-enzyme and only exists as a secreted protein [30].

Our understanding of the structural and functional diversity of NPPs has increased considerably in the last few years. This review deals primarily with the domain structure and enzymatic properties of NPPs, and with their putative role in the modulation of purinergic signaling, in particular in relation to epithelial and neural functions, the immune response and cell motility. The reader can find complementary information on NPPs in other recent reviews [31, 32].

Domain structure and catalytic properties of NPPs

NPPs are modular proteins (Figure 3). In addition to a catalytic domain they also contain subcellular targeting or anchoring domains as well as regulatory domains.

The catalytic domain: The NPP signature

All NPPs have a catalytic domain of about 400 residues that shows up to 60% identity at the amino acid level between the different human isoforms. This catalytic domain is predicted to adopt a fold similar to that of alkaline phosphatases, phosphopentomutases and cofactor-independent phosphoglycerate mutases, which all belong to the superfamily of phospho-/sulfo-coordinating metalloenzymes [33, 34]. Also, the residues that coordinate two metals in the catalytic site of alkaline phosphatases and their spatial arrangement towards the catalytic site, are conserved in NPPs. Furthermore, the reaction mechanism of NPPs appears to be similar to that of the other phospho-/sulfo-coordinating metalloenzymes and involves a two-step mechanism [18]. In the first step, the catalytic-site threonine/serine forms an intermediate with a phosphate group

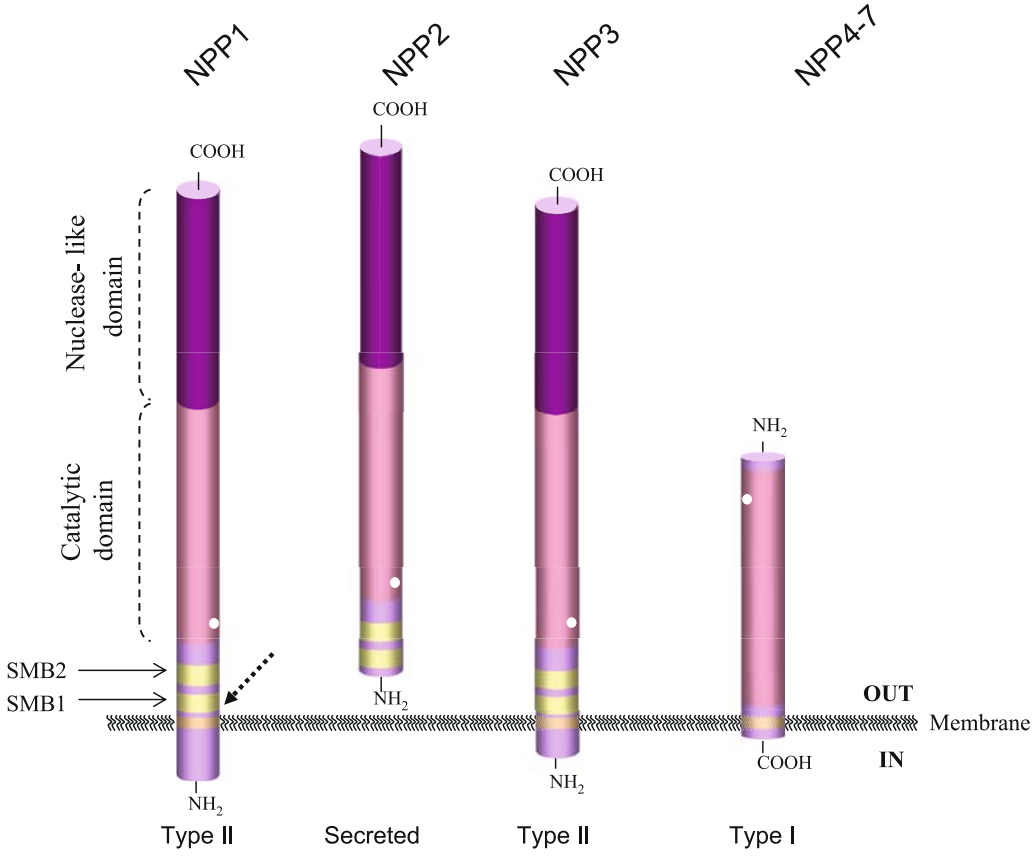

Figure 3. Domain structure and subcellular localisation of NPPs. Except for NPP2, which is secreted in the extracellular medium, NPP ectoenzymes are single-span membrane proteins with type-II (NPP1 and NPP3) or type-I orientation (NPP4–7). In all cases, the bulk of the protein lies outside the cell, with only short fragments facing the cytosol. Soluble NPP1 can be generated by cleavage of the membrane-associated form (*arrow*). The intracellular domains of NPP1 and NPP3 contain determinants for targeting to the basolateral or apical side of the plasma membrane, respectively. The common structural element of NPPs is the catalytic domain. Aminoacid identity of the catalytic domain, as obtained by Blast-2 sequence analysis of the human isoenzymes at the NCBI site is between 24% (NPP2–NPP6) and 60% (NPP1–NPP3). The position of the threonine/serine that mediates the formation of the catalytic intermediate is marked with *white circles*. The nuclease-like domain and the two somatomedin B-like domains (SMB1 and SMB2) are found only in NPP1–3.

of the incoming substrate. In the second catalytic step, a water molecule is used to regenerate the catalytic-site threonine/serine and to release the phosphorylated product. For example, ATP is hydrolyzed into either AMP + PP_i or ADP + P_i, via an AMP- or phosphate-bound intermediate, respectively, depending on how the substrate approaches the catalytic site [18].

The N-terminus: Signal peptide or signal anchor?

The N-terminal 21–76 residues harbour the determinants for the subcellular localization of human NPPs. In NPP1 and NPP3 this region functions as a signal anchor, ensuring a type-II orientation across the membrane, with the N-terminus facing the cytosol. In the polarized epithelial cells, NPP1 and NPP3 are expressed at different sides of the plasma membrane. A di-leucine motif in the intracellular domain targets NPP1 to the basolateral membrane [35]. The N-terminus of NPP3 does not contain this motif and is therefore targeted apically, in part after it transiently reaches the basolateral area [36]. The first 27 residues of NPP2 function as a signal peptide that is removed co-translationally by the signal peptidase in the endoplasmic reticulum. The resulting soluble pro-NPP2 is further cleaved by furin-type proteases along the secretory pathway to generate the mature, fully active NPP2 [30]. The N-terminus of NPP6–7 comprising about 20 residues functions as a signal peptide that is cleaved co-translationally. However, NPP6–7 have a C-terminal transmembrane domain, which accounts for their type-I transmembrane orientation, leaving the bulk of the protein extracellularly.

The somatomedin-B like domains of NPP1–3: An elusive function

Two cysteine-rich somatomedin-B like domains lie between the transmembrane and the catalytic domain of NPP1–3. One suggested function has been that of a 'stable stalk' between the transmembrane and the catalytic domains [10]. Also, NPP1 is a disulfide-linked homodimer and cysteines in these domains have been proposed to participate in interchain S–S bridges [18, 37]. However, the crystal structure of the somatomedin-B domain of vitronectin revealed that this domain forms a 'disulfide-knot', with all eight cysteines engaged in intrachain disulphide bonds [38]. This strongly indicates that the determinants for the dimerization of NPP1 lie outside the somatomedin-B like domains. Consistent with this view, secreted NPP2 does not form dimers although it also has two somatomedin-B like domains [30]. It is therefore likely that in NPP1–3 these domains function as protein interaction domains, similarly to those in vitronectin [38].

The C-terminal portion of NPP1–3: A multifunctional, nuclease-like domain

A stretch of about 250 residues that flanks the catalytic domain of NPP1–3 C-terminally shows similarities with DNA/RNA non-specific endoribonucleases, both with respect to its primary structure and the predicted fold [37]. This 'nuclease-like' domain does not endow NPPs with a nuclease activity because the residues that are essential for catalysis by endoribonucleases are not conserved in NPP1–3. However, the nuclease-like domain does appear to contain isoform-specific determinants for catalysis by NPP1 and NPP2, since the swapping of their nuclease-like domain results in an inactive NPP2, while NPP1 with the nuclease-like domain of NPP2 is fully active [39]. The nuclease-like domain also appears to be essential for the translocation of NPPs from the endoplasmic reticulum to the Golgi-apparatus, possibly because it is required for the correct folding of NPPs [37]. Finally, recent evidence indicates that the nuclease-like domain of NPP2 has an anti-adhesion function and may function as a ligand for a G-protein coupled receptor [40].

The substrate specificity of NPPs: Many unanswered questions

NPPs can hydrolyze pyrophosphate or phosphodiester bonds in a wide variety of substrates, although each isoform has a well-defined substrate specificity (Figure 1). For example, NTPs and diadenosine polyphosphates are substrates for NPP1-3, while lysophosphatidylcholine is only hydrolyzed by NPP2 and NPP6. Remarkably, NPP2 and NPP6 hydrolyze distinct phosphodiester bonds in lysophosphatidylcholine, generating choline and phosphocholine, respectively. The determinants of the substrate specificity of NPPs are poorly understood. Clearly, the pyrophosphate or phosphodiester bond is not the only substrate-specifying element of NPPs. Site-directed mutagenesis and domain swapping studies revealed that the non-catalytic domains as well as residues near the catalytic site control the activity and substrate specificity of NPP1 and NPP2 [39]. The catalytic domain of NPP1 contains a GxGxxG motif that resembles part of a consensus dinucleotide-binding motif and is essential for catalysis by NPP1, but it does not appear to be a substrate-specifying motif [39]. Lipid-consensus motifs, similar to those found in phospholipases D are not present in NPP2. [41]. Nevertheless, it cannot be excluded that NPPs contain hitherto unrecognized nucleotide or lipid binding sites similarly to, for example, the substrate-specifying 'pockets' of serine proteases.

Role of NPP1–3 in the metabolism of extracellular nucleotides

NPP1–3, alone or in combination, are expressed in every cell type that has been analyzed for their presence [18, 31]. It therefore seems likely that these NPPs fulfill a broad range of functions. NPP isozymes may even 'moonlight,' in that they can fulfill multiple, apparently unrelated functions. For example, NPP1 is not only implicated in nucleotide metabolism but also appears to have an anti-insulin action by a mechanism that does not require catalytic activity [42, 43]. By far the best studied NPP

function concerns the role of NPP1 in bone and soft-tissue mineralization [44, 45], which is the subject of a separate review in this issue. We only discuss here how NPP1–3 contribute, as nucleotide-metabolizing enzymes, to the epithelial and neural functions, the immune response and cell motility.

NPP1–3 and epithelial functions

Most epithelial cells release nucleotides, either constitutively or when challenged with stimuli like mechanical stress or hypotonicity-induced swelling. In the extracellular environment of epithelial cells, purinergic signaling is of prime importance for ion transport, cell–cell communication and cell migration [46–48].

Airway epithelia

Nucleotide metabolism at the surface of human airways has received much attention because of the critical role of nucleotides in the protective mechanism against bacterial and viral infections, known as mucociliary clearance (MCC) [49–53]. This defense mechanism involves complex signaling by diadenosine polyphosphates, ATP and adenosine, affecting overall fluid homeostasis via a control of epithelial chloride secretion and sodium uptake. In fact, nucleotide-based treatments combined with ecto-nucleotidase inhibitors are used for the improvement of MCC in chronic obstructive lung diseases such as cystic fibrosis.

Some efforts have been undertaken to identify the ectonucleotidases expressed by airway epithelia. There is good biochemical evidence for the presence of both NTPDase-type [51] and NPP-type activities [54] in the airway epithelia. Transcript analysis revealed the presence of various ectonucleotidases including NTPDase2, NTPDase3, NTPDase5 as well as NPP1–3 [55]. However, not all of them may be expressed at the protein level equally well. Importantly, human airway ectonucleotidases are cell-associated and are predominantly apical [51]. Within the NPP family, this suggests a role for NPP3, which is indeed targeted to the apical surface, while NPP1 is expressed at the basolateral one, and NPP2 only exists as a secreted form. However, NPP2 might also fulfill a critical function in the lung because the transcript level in the lung is among the highest of all tested organs [56]. In airways an NPP-type pyrophosphatase activity may be mainly involved in regulation of signaling by diadenosine polyphosphates, which were recently shown to bind to P2Y purinergic receptors distinct from those activated by ATP [57]. Other ectonucleotidases identified in human airway epithelia are the tissue non-specific alkaline phosphatase, exclusively expressed at the apical surface, and 5′-nucleotidases, expressed at both apical and basolateral sides [58].

Liver epithelia

The liver epithelial cells, i.e. hepatocytes and cholangiocytes, contribute to bile formation and secretion, one of the primary functions of the liver. Bile is formed by the parenchymal hepatocytes and is further enriched in bicarbonate by cholangiocytes, the cells that line the bile ducts [59, 60]. The biliary epithelium constitutively releases ATP into the bile from a vesicular storage site, and increase in cell volume promotes the exocytosis of ATP through a phosphoinositide 3-kinase-dependent mechanism [61]. Hepatocytes, too, contribute to the biliary pool of ATP [59]. NPP3 is the major NPP isoenzyme at the apical membrane of both hepatocytes and cholangiocytes [62, 63]. An apical localization in hepatocytes has also been established for 5′-nucleotidase [59]. By regulating the breakdown of biliary ATP these nucleotide-hydrolyzing enzymes may interfere with purinergic signaling at the bile canaliculi, and thereby modulate the process of bile formation. In cholangiocytes, NPP3 may be one of the major apical ectonucleotidases, consistent with the functional expression of a single, dominant ATP degradation pathway at this site [64]. By contrast, NTPDase2/CD39L1 has been identified in portal fibroblasts and appears to be the main ectonucleotidase that indirectly controls purinergic signaling at the basolateral membrane of cholangiocytes [65]. The role of basolateral nucleotides in bile ductular secretion is relatively minor when compared to that of apical nucleotides, but there is evidence that basolateral nucleotides modulate the growth and differentiation of cholangiocytes [66].

The role of NPP3 in cholangiocytes may extend beyond the regulation of bile formation. Indeed, large amounts of NPP3 are found in the extracellular matrix and in the serum of chemically induced rat cholangiocarcinoma [67], an adenocarcinoma of intrahepatic bile ducts. Neoplastic transformation of biliary cells leads to an overexpression of NPP3 but also to its inappropriate targeting to the basolateral rather than to the apical side of the cholangiocytes. Since NPP3 can stimulate migration of cultured cells it may also be involved in metastasis of neoplastic cholangiocytes, by an as yet unknown mechanism [63].

NPP1 is also abundantly expressed in hepatocytes and localizes basolaterally [63]. An enticing hypothesis is that this pool of NPP1 controls the turnover of extracellular ATP, which functions as a potent hepatic mitogen by increasing immediate early gene expression in a P2-receptor dependent manner [68]. Consistent with this notion, the level of NPP1 is considerably decreased during liver regeneration following 70% partial hepatectomy and only reaches its initial level again after the major growth phase of the liver has passed [69]. We speculate that the transient drop in the concentration of NPP1 enables the accumulation of extracellular ATP, needed to stimulate cell proliferation.

Intestinal epithelia

Enterocytes constitute the major fraction of intestinal epithelial cells and their primary function is to absorb nutrients from the diet. Nucleotides are semi-essential or conditionally essential nutrients [70]. Cells can synthesize nucleotides de novo and re-use them through the nucleotide salvage pathway. However, rapidly proliferating tissues such as the intestine have an insufficient biosynthetic capacity and are dependent on an exogenous supply

of nucleotides. NPP3 is the major NPP isoenzyme at the brush border of enterocytes [62]. Given its very high expression, combined with its co-localization with 5′-nucleotidase [71] or alkaline phosphatase, it seems likely that these ectonucleotidases play an important role in the digestion of dietary nucleotides and their derivatives but a direct evidence for this contention is lacking.

NPP1–3 and neural functions

Extracellular nucleotides, in particular ATP, have multiple functions in the central nervous system. ATP acts as a neurotransmitter and modulator of neurotransmitter release, but also as a trophic factor that stimulates proliferation and differentiation of neural cells [5, 72–75]. Neurons, glial cells and endothelial cells are established sources of extracellular purines. Initial evidence reveals important functions for NPPs in the nervous system, at least in part by their ability to modulate purinergic signaling.

Within the brain, a panel of P2X receptors as well as NPP2 and NPP3 are expressed by the choroid-plexus epithelial cells [76–78]. The latter cells secrete cerebrospinal fluid (CSF), the major extracellular fluid in the central nervous system. CSF provides the central nervous system with nutrients and signaling molecules, and removes metabolites [79]. It seems likely that NPP2/3 contribute to the secretion of CSF by modulating purinergic signaling [78]. In rodents NPP2 can also be detected in the CSF itself. This pool of NPP2 possibly originates from the choroid-plexus epithelial cells and/or from the leptomeningeal cells and has recently been shown to function as a neurite retraction factor [80]. Remarkably, in humans NPP2 is absent from the CSF of healthy persons, but is found in the CSF from patients with multiple sclerosis [81].

NPP2 is also secreted by oligodendrocytes, the myelin producing cells [82]. A tight correlation was noted between the expression of NPP2 in oligodendrocytes and the initial stages of myelination, a complex process that includes the movement of oligodendrocytes to the sites of myelination. NPP2 has been identified as an autocrine ‘counteradhesive’ factor and may thus be implicated in the movement of oligodendrocytes to their target sites [82]. Surprisingly, this function does not require the catalytic domain of NPP2 and appears to be mediated by the nuclease-like domain, which possibly interacts with a G-protein coupled receptor [40].

In the developing brain NPP3 is first scattered throughout the neuroepithelium and adjacent mesenchymal tissue, but later appears in distinct brain regions to reach the strongest expression on meninges, ependymal layers and choroid plexus [77]. The latter structures maintain a strong expression of NPP3 until adulthood. NPP3 was also found to be transiently expressed in a population of neural progenitor cells that can differentiate into radial glial-like cells, a subset of astrocytes and ependymal cells [77]. Since NPP3 is not expressed by mature astrocytes, this may suggest an involvement of this isoenzyme in keeping their progenitor cells dedifferentiated. Consistent with this notion, it was reported that the expression of NPP3 in NPP3-negative cell lines induces the expression of glial fibrillary acidic protein, an intermediate-filament protein specific for the cytoskeleton of astroglial cells and non-myelin-forming Schwann cells. Interestingly, the overexpression of NPP3 has also been associated with increased motility and invasive properties of cultured cells [83].

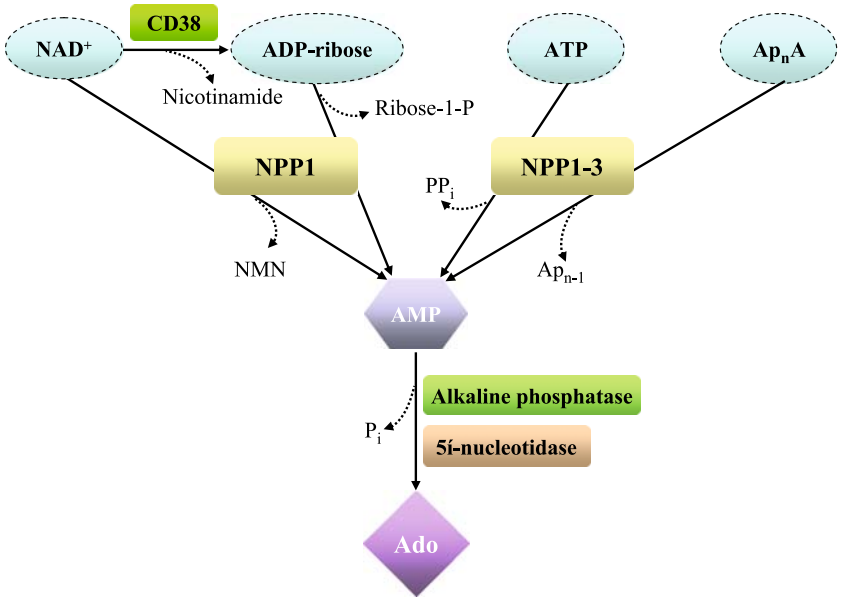

Figure 4. Role of NPP1–3 in the metabolism of extracellular nucleotides. NPP1 and CD38 are co-expressed in T-lymphocytes. Extracellular NAD^+ is a substrate for NPP1, a NAD^+-pyrophosphatase, as well as for CD38, a NAD^+-glycohydrolase. The coordinated expression of NPP1 and CD38 is part of a protective mechanism against NAD^+-induced apoptosis of T cells. The hydrolysis of NAD^+ by the concerted action of NPP1, CD38 and 5′-nucleotidase also allows activated T cells to re-use the products for their own anabolic processes. At the apical membrane of hepatocytes and cholangiocytes, NPP3 hydrolyzes bile ATP and modulates purinergic signaling at these sites. Diadenosine polyphosphates ($Ap_{3-5}A$) and other nucleotides are hydrolyzed at the apical surface of the human airways by an NPP-type pyrophosphatase. Likely candidates are NPP3 and/or NPP2.

Expression of NPP1 in glia cells under physiological conditions has not been reported. However, NPP1 is the major ectonucleotidase in the rat C6 glioma cell line [84, 85]. This cell line is morphologically similar to glioblastoma multiforme, the most common aggressive glioma resistant to therapeutic interventions [86].

NPP1 and lymphocyte signaling

NPP1 is a marker of a late stage in the differentiation of antibody-producing B cells, but its expression is not required for the synthesis and secretion of antibodies. Although initially believed to be lineage-specific [87], in the meantime it has been established that low levels of NPP1 are also found in T cells and that its expression in these cells is increased through signaling by protein kinases A and C, the same pathways that also up-regulate the expression of the NAD^+ glycohydrolase CD38 [88]. The coordinated expression of NPP1 and CD38 enables the hydrolysis of extracellular NAD^+ by activated T cells to nicotinamide mononucleotide + AMP or to nicotinamide + ADP-ribose, respectively (Figure 4). In doing so, NPP1 and CD38 control the substrate availability for NAD^+-dependent ADP-ribosylation by ADP-ribosyltransferases at the cell surface. This can be seen as a protective mechanism against apoptosis at sites of inflammation, where the release of NAD^+ results in ADP-ribosylation and activation of the pro-apoptotic $P2X_7$ receptors [89, 90]. By the same mechanism NPP1 and CD38 also provide a protection against the ADP-ribosylating bacterial toxins, such as cholera or *C. botulinum* C3 toxins, which exert their cytotoxic effects through ADP-ribosylation of host signaling molecules [91]. Both NAD^+ and its degradation product ADP-ribose are potent inhibitors of T-cell proliferation [92]. Their hydrolysis by CD38 and NPP1 is thus expected to promote T-cell proliferation. Finally, in combination with 5′ nucleotidase, CD38 and NPP1 are part of the nucleotide-salvage pathway [88] (Figure 4).

NPP2 and cell motility

NPP2 is secreted by various cancer cells including skin, lung and breast cancer cells. Its tumour growth and motility effects have been primarily attributed to its ability to produce lysophosphatidic acid (LPA) from lysophosphatidylcholine (LPC) [93]. LPA binds to dedicated G-protein-coupled receptors, $LPA_{1–4}$, and activates multiple signaling pathways, leading to cell proliferation, cell-shape changes and migration. These effects explain why NPP2 promotes tumour growth, angiogenesis and metastasis (Figure 5). However, it can be envisaged that NPP2 also promotes tumour growth by its ability to hydrolyze nucleotides. Indeed, solid tumours are well known to release adenine nucleotides and NPP2 can hydrolyze ATP, which is an inhibitor of tumour-cell proliferation [49]. Moreover, the concerted action of NPP2 and 5′-nucleotidase on ATP and ADP generates adenosine, a tumour-growth promoter and stimulator of angiogenesis (Figure 4) [94–96]. In this respect, it is also worthy of note that the Wnt/β-catenin signaling pathway is activated in many human tumours, resulting in an increased expression of both NPP2 [97, 98] and 5′-nucleotidase [99].

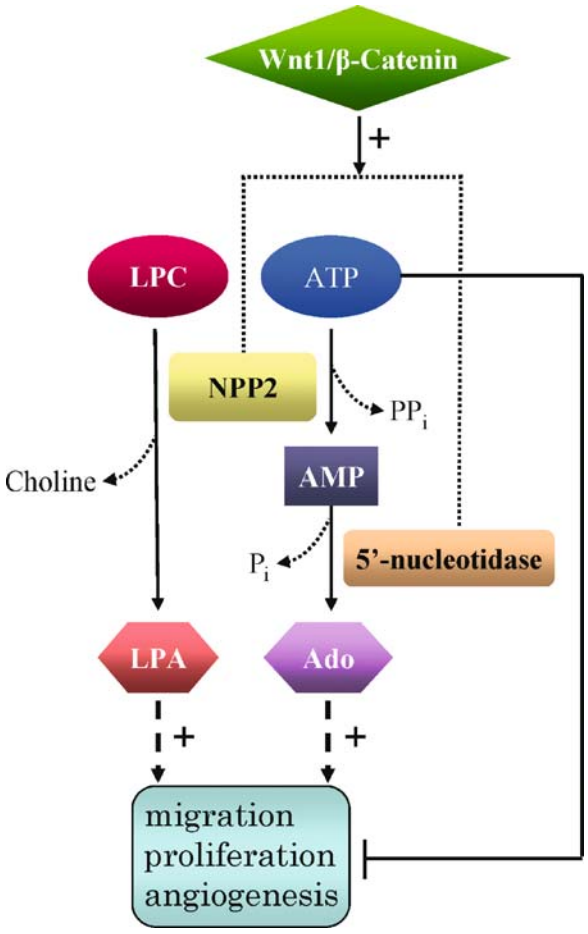

Figure 5. Tumour growth and metastasis: Role of NPP2-catalyzed reactions. In the extracellular milieu the tumour-motility stimulating factor NPP2 generates LPA from LPC. LPA promotes cell proliferation, migration and angiogenesis. In a coupled reaction with 5′-nucleotidase, NPP2 has the potential to hydrolyze ATP, which is cytotoxic for tumours, to adenosine, a tumour-growth promoting factor and stimulator of angiogenesis. The expression of both NPP2 and 5′-nucleotidase is increased by the Wnt/β-catenin pathway, which is activated in many cancers.

Conclusions and perspectives

The family of NPP-type ectophosphodiesterases comprises a versatile group of seven ecto-enzymes that hydrolyze pyrophosphate and phosphodiester bonds in a broad range of metabolites, yet each isozyme has a rather narrow substrate-specificity. Only NPP1–3 are known to hydrolyze nucleotides and they are part of a complex nucleotide interconversion network at the cell surface. NPP1–3 contribute to ectonucleotide signaling both by removing active compounds and by generating nucleotide metabolites with distinct signaling properties. The complexity of signaling via NPPs is further increased by their ability to hydrolyze non-nucleotide substrates. For example, NPP2 can hydrolyze both phospholipids and nucleo-

tides and the generated products have the potential of acting antagonistically or synergistically. A surprising finding has been that NPP1 and NPP2 have also functions that do not require catalytic activity. This can imply that NPPs moonlight, although it cannot be ruled out that their noncatalytic functions are somehow connected to their catalytic functions.

Much remains to be learned about the enzymatic properties, regulation and functional diversity of NPPs. A lot is expected from the phenotyping of knockout models for each NPP isoenzyme. Also, the availability of isoenzyme-specific NPP inhibitors would be of great help to study their function. The latter also have therapeutic potential as they hold great promises for the treatment of devastating diseases like cystic fibrosis and bone-mineralization pathologies.

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