Dear author,

Please note that changes made in the online proofing system will be added to the article before publication but are not reflected in this PDF.

We also ask that this file not be used for submitting corrections.

Trends in Molecular Medicine

CellPress

Review

MicroRNAs Modulate the Purinergic Signaling Network

Q1 Davide Ferrari,[1,*] Nicoletta Bianchi,[1] Holger K. Eltzschig,[2] and Roberto Gambari[1]

MicroRNAs (miRNAs) are small non-coding RNA molecules capable of silencing mRNA targets. miRNA dysregulation has been linked to cancer development, cardiovascular and neurological diseases, lipid metabolism, and impaired immunity. Therefore, miRNAs are gaining interest as putative novel disease biomarkers and therapeutic targets. Recent studies have shown that purinergic surface receptors activated by extracellular nucleotides (ATP, ADP, UTP, UDP), and by nucleosides such as adenosine (ADO), are subject to miRNA regulation. This opens a new and previously unrecognized opportunity to modulate the purinergic network with the aim of avoiding abnormal activation of specific receptor subtypes. miRNA technology will hopefully contribute strategies to prevent purinergic-mediated tissue damage in conditions of neurodegeneration, atherosclerosis, transplantation, and perhaps even neoplasia.

The Purinergic Signaling Network

Nucleotides (ADP, ATP, UDP, UTP, GTP) play fundamental roles in living cells as monomers that build nucleic acids, as energy carriers in metabolic pathways, and as components of various coenzymes [1]. However, nucleotides and nucleosides such as ADO can exhibit completely different functions in the extracellular space where they are released. They behave as mediators of cell-to-cell communication by binding to and activating specialized plasma-membrane receptors expressed across species, and by virtually all tissues [2,3]. Receptors for extracellular nucleotides, named P2 receptors, are divided into two subgroups: P2Y and P2X receptors [4–6]. P2Y receptors are G protein-coupled seven transmembrane domain receptors, with an extracellular N-terminus and an intracellular C-terminus. Eight human P2Y subtypes have been cloned and pharmacologically characterized. They have been named P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14. In addition to differences in gene sequence, they also show heterogeneity in G protein coupling, agonist specificity, and activation of intracellular signaling cascades. ADP activates P2Y1, P2Y12 and P2Y13, while UDP is an agonist at P2Y6. UTP binds to and activates P2Y2 and P2Y4, and to a lesser extent P2Y6, while the other P2Y subtypes are activated by ATP [5]. P2X receptors are ionotropic ATP-activated receptors that trimerize, forming ion channels selective for monovalent and divalent cations such as Na^+, K^+, Ca^{2+}, and Mg^{2+}. Seven receptor subunits have been identified and numbered from 1 to 7 [6]. P2X1 can form heterotrimers with P2X2, P2X4, and P2X5 subunits, while P2X2 forms hetero-oligomers with P2X3 [6].

By contrast, ADO receptors are referred to as P1 receptors; they are G protein-coupled receptors with seven transmembrane domains and can be divided into four subtypes known as A_1, A_{2A}, A_{2B}, and A_3 receptors [7]. They show different affinities for the agonist ADO, and are diverse in their recruitment of G proteins, as well as in the activation of specific intracellular signaling cascades [7].

Trends

Nucleotides and nucleosides play multiple functions within a cell. However, they behave as signaling molecules once released extracellularly, where they bind to specific cell-membrane purinergic receptors. ATP, ADP, UTP, UDP, and UDP-glucose activate P2 receptors, while ADO activates P1 receptors.

Plasma-membrane ectonucleotidases CD39 and CD73 convert ATP/ADP to AMP, and AMP to ADO, respectively, thus regulating the concentration of P2 and P1 receptor agonists and consequent responses. Adenosine deaminase (ADA) terminates P1-mediated signaling by inactivating ADO.

miRNAs are small RNA molecules regulating gene expression by silencing mRNA targets. They exhibit great regulatory potential during organismal development, cell proliferation and death, hematopoiesis, and immunity. miRNA dysregulation has been implicated in many pathologic states including cancer, neurological diseases, and metabolic disorders.

miRNAs modulate the expression of P1 and P2 purinergic receptors, as well as of ectonucleosidases CD39 and ADA2. Q2 miRNAs can thus affect the global outcome of purinergic responses, influencing many physiological and pathological processes governed by extracellular nucleotides/nucleosides.

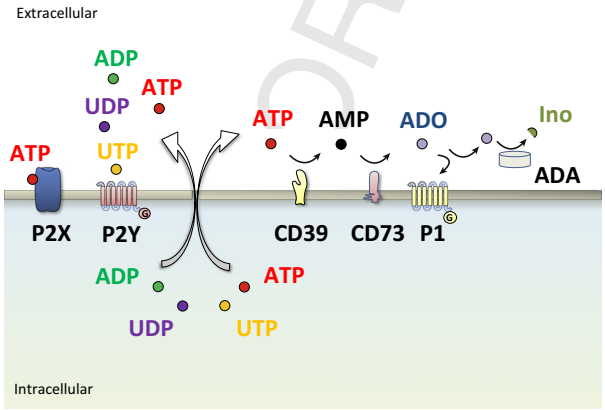
[1]Department of Life Science and Biotechnology, University of Ferrara, Ferrara, Italy
[2]Department of Anesthesiology, University of Texas Medical School at Houston, Houston, TX, USA

*Correspondence: davide.ferrari@unife.it (D. Ferrari).

Extracellular ADO is generated by the sequential activation of plasma-membrane ectonucleotidases: ectonucleoside triphosphate diphosphohydrolase (NTPDase or CD39), ecto-5'-nucleotidase (CD73), ectonucleotide pyrophosphatase/phosphodiesterase, and alkaline phosphatases [8,9]. ADO can either be transported back into the cytoplasm or degraded extracellularly by the enzymes adenosine deaminase (ADA) or aminohydrolase, generating inosine that is inactive at P1 receptors (Figure 1).

Purinergic receptors modulate many biological functions ranging from heart rate, vascular tone, neuronal excitation, tissue repair, and immune responses in humans as well as in other species [10–14] (Figure 1). Specialized plasma-membrane molecules, such as connexin hemichannels, pannexin channels, the P2X7 receptor, **ATP-binding cassette (ABC) transporters** (see Glossary), and ATP-conducting anion channels, allow the release of ATP into the extracellular milieu [15–17], while extracellular ADO is generated by the sequential activation of plasma-membrane ectonucleotidases [8,9]. Uncontrolled ATP liberation such as that resulting from plasma-membrane mechanical stress and trauma, or derived from organismal allergen inhalation, and infection, can cause overactivation of P2X and P2Y receptors, with excessive production of inflammatory mediators [prostaglandins, reactive oxygen intermediates (ROIs), proinflammatory cytokines] [18–20]. Subsequently, massive recruitment of immune cells into inflamed tissues can ensue and favor the establishment of chronic inflammation [18–20].

Neuronal degeneration, chronic pain, metabolic dysfunction, atherosclerosis, thrombosis, allergy, asthma, and even cancer have all been linked to dysregulated events in the purinergic signaling network [21–27]. P2Y12 receptor antagonists such as clopidogrel and ticagrelor have been successfully used for antithrombotic therapy in patients suffering from heart attacks or other circulatory problems [28,29]. With respect to therapeutic approaches, although stimulation or inhibition of specific P1 or P2 receptors has become a new opportunity to treat different pathologies, many other human diseases such as rheumatoid arthritis, Alzheimer's and Huntington's diseases, multiple sclerosis, and **epilepsy** have no real cure and thus await therapies that might facilitate normalizing the dysregulation of purinergic signaling (if pertinent) to potentially



Trends in Molecular Medicine

Figure 1. Nucleotide and Nucleoside Signaling at the Plasma Membrane. Nucleotides (ATP, ADP, UTP, UDP) and nucleosides (ADO) are released by the cell into the extracellular milieu where they can be degraded or bind to specific purinergic receptors named P1 and P2 receptors. P1 receptors are activated by adenosine (ADO), while P2 receptors are activated by ATP (P2X) and/or ADP, UTP, etc. (P2Y). The fate of ATP and ADP depends on expression of plasma-membrane ectonucleotidases (CD39 and CD73) that sequentially transform ATP/ADP to AMP and ADO. ADO is irreversibly inactivated to inosine by adenosine deaminase (ADA).

Glossary

A_{2A} receptor: also known as ADORA2A, a purinergic receptor belonging to the P1 group. Activated by adenosine (ADO), it is involved in cardiac rhythm and the regulation of blood circulation. It can also modulate immune function, pain sensation, and sleep.

ATP-binding cassette (ABC) transporters: plasma-membrane proteins that couple energy derived from ATP hydrolysis to the efflux of a variety of molecules from the cell, including xenobiotics.

Actinomycin D: antibiotic produced by *Streptomyces* spp. It interferes with elongation of RNA chain during transcription by binding to pre-melted DNA.

Amyotrophic lateral sclerosis (ALS): disease characterized by gradual degeneration and death of motor neurons of the brain and spinal cord. It can be either sporadic or familial, and is characterized by rapid progression and fatal outcome. Although treatment can slow progression of the disease in some subjects, a cure is not yet available.

Cationic lipoplexes: cationic lipid/nucleic acid complexes that are synthetic amphiphilic molecules with hydrophilic and hydrophobic regions mimicking the cell membrane. Used as a non-viral delivery system for nucleic acids (including miRNAs or other molecules employed in RNA interference).

Cell-penetrating peptides (CPPs): formed by a restricted number of amino acids, they are used to improve the delivery of bioactive molecules such as miRNAs into the cell.

Epilepsy: chronic neurological disorder due to excess electrical activity in the brain: it is characterized by seizures and periods of unusual behavior and sensations, sometimes culminating in loss of consciousness.

Erythrocyte ghosts: red blood cells whose intracellular content has been replaced with bioactive molecules such as miRNAs. They are used as a delivery system for clinical purposes.

Graft-versus-host disease (GVHD): possible pathological consequence of tissue transplantation. It is characterized by a toxic immunological reaction by immune cells present in the transplanted tissue (graft) that is directed against host tissues.

ameliorate patient conditions. The present knowledge that purinergic receptors and ectonucleotidases can be under the control of miRNAs opens up the possibility of modulating their transcription by applied miRNA technologies. We summarize recent findings that advance our current understanding of the role of miRNAs in influencing the expression of single molecular components of the purinergic signaling network. This knowledge also lays down a platform to envisage the therapeutic potential of modulating miRNA activity by purinergic stimulation for various pathologies.

miRNAs: Promising Tools for New Therapies

miRNAs are widely present in Metazoa and are endowed with the ability to silence mRNA targets to regulate gene expression during various processes including, but not limited to, organismal development, cell proliferation, cell death, hematopoiesis, and immunity [30–34]. miRNAs are a family of small (19–25 nt in length) noncoding single-stranded RNAs that post-transcriptionally regulate gene expression by sequence-selective targeting of mRNAs, leading to translational repression or mRNA degradation, depending on the degree of complementarity between miRNAs and target mRNA sequences [35,36]. Intracellular miRNA levels depend on different factors that modulate their expression, biogenesis, and degradation. They usually have a longer lifespan than mRNAs; however, the situation may change depending on the type of miRNA and the cell context. RNases are the intracellular enzymes responsible for miRNA degradation. Although multiple miRNAs have been identified, a deeper investigation of the biochemical mechanisms regulating their function is needed. It has been shown that the presence of specific nucleotides in miRNA sequences affects their stability. For instance, three uridines at positions 9–11 in miR-29b have been found to favor its decay [37].

Owing to their importance as regulatory molecules, and to the availability of new and effective tools to screen for the presence of single or pathway-associated miRNAs, they have received increasing attention, and a growing number of miRNA sequences have been deposited in miRBase databases One example is the UCbase & miRfunc database, which functionally describes miRNAs and their relationship to human disorders (http://microrna.osu.edu/.UCbase4) [38].

Q3 It is calculated that at least 10–40% of human mRNAs are targets for miRNAs [30], opening previously unsuspected opportunities for modulating eukaryotic signal transduction of structural and regulatory proteins that control fundamental biological functions such as cell proliferation, differentiation, and death [36,37].

Many *in vivo* studies with different animal models have highlighted the possibility of identifying miRNA dysregulation as a diagnostic marker for specific pathologies. Moreover, the efficacy of miRNAs as potent modulators of the expression of genes involved in different diseases has been noted [33,39]. For instance, cancer overall holds a prominent position among the pathologies that might be potentially treated via miRNAs [40]. Nevertheless, a growing number of other diseases and conditions are regularly being added to this targeting list (and comprising those affecting humans); these include viral infections, neuronal dysfunctions, and chronic inflammatory diseases [41–43]. In the past few years the identification of miRNAs playing a role in specific pathologic contexts has been paralleled by a deep investigation of specific cell delivery approaches of miRNA-mimicking or miRNA antagonist molecules (antagomiRNAs). Some of the utilized or tested delivery technologies include the use of **erythrocyte ghosts**, **cationic lipoplexes**, **neutral lipid emulsions** (NLEs), **nanoparticles**, and **cell-penetrating peptides** (CPPs), many of which have been recently proposed and validated [44–47]. Bacteriophages have also been used to develop virus-like particles that can be coupled to oligonucleotide delivery [48]; however, because these can stimulate host immune responses, further studies are

Locked nucleic acid (LNAs): chemically synthesized modified oligonucleotides which efficiently and specifically hybridize with other nucleic acid molecules to produce a stable duplex. LNA technology is applied to DNA microarrays, FISH, quantitative PCR, *in situ* detection of miRNAs, and the development of therapeutic oligonucleotides.
Luciferases: enzymes widely used in bioluminescence assays to measure a variety of biological parameters and functions.
miRNA masking: inhibition of miRNA function by covering miRNA binding sites with a modified single-stranded RNA complementary to the target sequence.
miRNA replacement therapy: a new therapeutic approach aiming to correct low miRNA expression with synthetic miRNA mimetics, for example in some cancers.
miRNA sponges: a molecular biology approach used to generate RNAs containing miRNA binding sites. miRNA sponges are synthetic RNAs containing multiple tandem binding sites for an miRNA family. Application of this technology allows *in vivo* suppression of classes of paralogous miRNAs.
Morpholinos: chemically modified oligonucleotides with a backbone of methylenemorpholine rings and phosphorodiamidate linkages. They block the activity of miRNAs by competing for their binding site on a target mRNA. They are also used for gene knockdown.
Nanoparticles: microscopic particles of less than 100 nm. They can be used for increasing drug or miRNA delivery into a cell and reduce systemic toxicity of bioactive compounds.
Neutral lipid emulsion (NLE): a mixture of lipidic molecules that can be complexed with miRNAs to favor cellular uptake. The complexes can be administered intravenously.
Nociceptive sensitivity: perception of pain by specialized neuronal cells (nociceptors) distributed throughout the body.
Peptide nucleic acid (PNA): synthetic oligomer in which deoxyribose and ribose sugars are substituted by repeating *N*-(2-aminoethyl)-glycine units. PNAs bind complementary nucleic acid molecules more strongly than canonic DNA or RNA probes. PNAs can be used as miRNA inhibitors and

certainly needed before clinical applications can be entertained. Recently, exosome-based transfection applications have also been introduced as delivery systems aiming to encapsulate synthetic miRNAs or antagomiRNAs, while concomitantly providing advantages of high biocompatibility and low toxicity [49].

Another example includes the early clinical data that have been obtained on **locked nucleic acid** (LNA)-modified DNA phosphorothioate antisense oligonucleotides (miravirsen); these have been used to inactivate specific miRNAs in patients with chronic hepatitis C virus (HCV) infection, with encouraging results. In this HCV study, miravirsen was able to sequester mature miR-122 in a highly-stable heteroduplex, thereby inhibiting its function in a prolonged and dose-dependent manner [50].

miRNAs: An Effective Way To Modulate Purinergic Signaling

Modulation of Purinergic Signaling

Molecular components of the purinergic signaling network can be either activated or inhibited in different ways [51–53]. As an example, large spectrum or specific P1 and P2 receptor agonists and antagonists have been made available and allow wide, restricted or punctual modulation of purinergic receptors [54–56]. Knockout (KO) mouse models have also been a valuable tool for discerning the role of single purinergic receptors and ectonucleotidases [57–60]. Abrogation of the expression of single receptor or nucleotide-degrading enzyme genes has provided insight into the physiological role of these pathways in mice, allowing functional speculation for humans. For example, P2X1 KO mice have been found to exhibit reduced vas deferens contraction and male infertility [57], while P2X7 KO mice have provided mechanistic insight into how P2X7-mediated signaling impacts on rod and cone pathway responses of the retina [58]. The P2Y12 KO mouse model has been used to study the involvement of this P2Y12 in modulating microglia migratory properties and neurotoxicity under ischemic conditions, or, alternatively, to investigate atherosclerotic pathological modifications [59,60]. Lastly, the CD73 KO mouse model has been used to document a protective role for this ectonucleotidase in **graft-versus-host disease** (GVHD) [61]. *In vitro* and *in vivo* experiments have also been used to reduce or block purinergic receptor activation indirectly, by depleting the extracellular milieu of receptor agonists (ATP, ADP, ADO) via degradation. For instance, apyrase, an ATP diphosphohydrolase which converts ATP to AMP and inorganic phosphate, has been reported to decrease extracellular ATP concentrations in cells for a few models, including canine pulmonary ischemia–reperfusion injury and arterial thrombosis [62–64]. Application of this strategy might be promising in the treatment of various pathologies, but further testing is required [62–64].miRNAs involved in the modulation of purinergic signaling might thus represent a future strategy for therapeutic intervention. Inhibition of miRNA activity (a strategy proposed for anti-miRNA therapy) can be achieved by the use of: (i) oligomer miRNA-based inhibitors, (ii) small-molecule inhibitors [LNAs, **peptide nucleic acids** (PNAs), **morpholinos**, **miRNA sponges**), or via (iii) **miRNA masking**. By contrast, increasing miRNA function might be used to offset downregulated miRNA levels found in cells or tissues for various pathologies – a strategy proposed for **miRNA replacement therapy**. This might be potentially achieved via modified miRNA mimetics (synthetic, or plasmid/lentiviral vector products) [65]. The multiplicity of different approaches has allowed the transient (miRNA mimetics) or stable (vector-mediated miRNA expression) production of putative therapeutic miRNAs to presumably replace abnormal microRNA functions. miRNAs are emerging as effective translational regulators of P1 and P2 receptors, particularly for A_{2A}, A_{2B}, and P2X7 receptors, and some purinergic network enzymes such as CD39 and ADA also seem to be regulated by miRNAs (Figure 2). For instance, recent data suggest that miRNAs modulate purinergic receptors in normal and transformed cells; in the following section Table 1 summarizes experimental evidence indicating that miRNAs can modulate molecular component(s) of the purinergic network.

are often conjugated to peptides to facilitate cell entry.

Regulatory T cells (T regs): a subpopulation of $CD4^+$ T lymphocytes involved in tolerance mechanisms as well as in the suppression and regulation of specific immune responses.

T helper 17 (Th17) lymphocytes: secrete IL-17 and are involved in adaptive immunity; Th17 cells are implicated in pathologic inflammation, autoimmune disorders, and tumor biology.

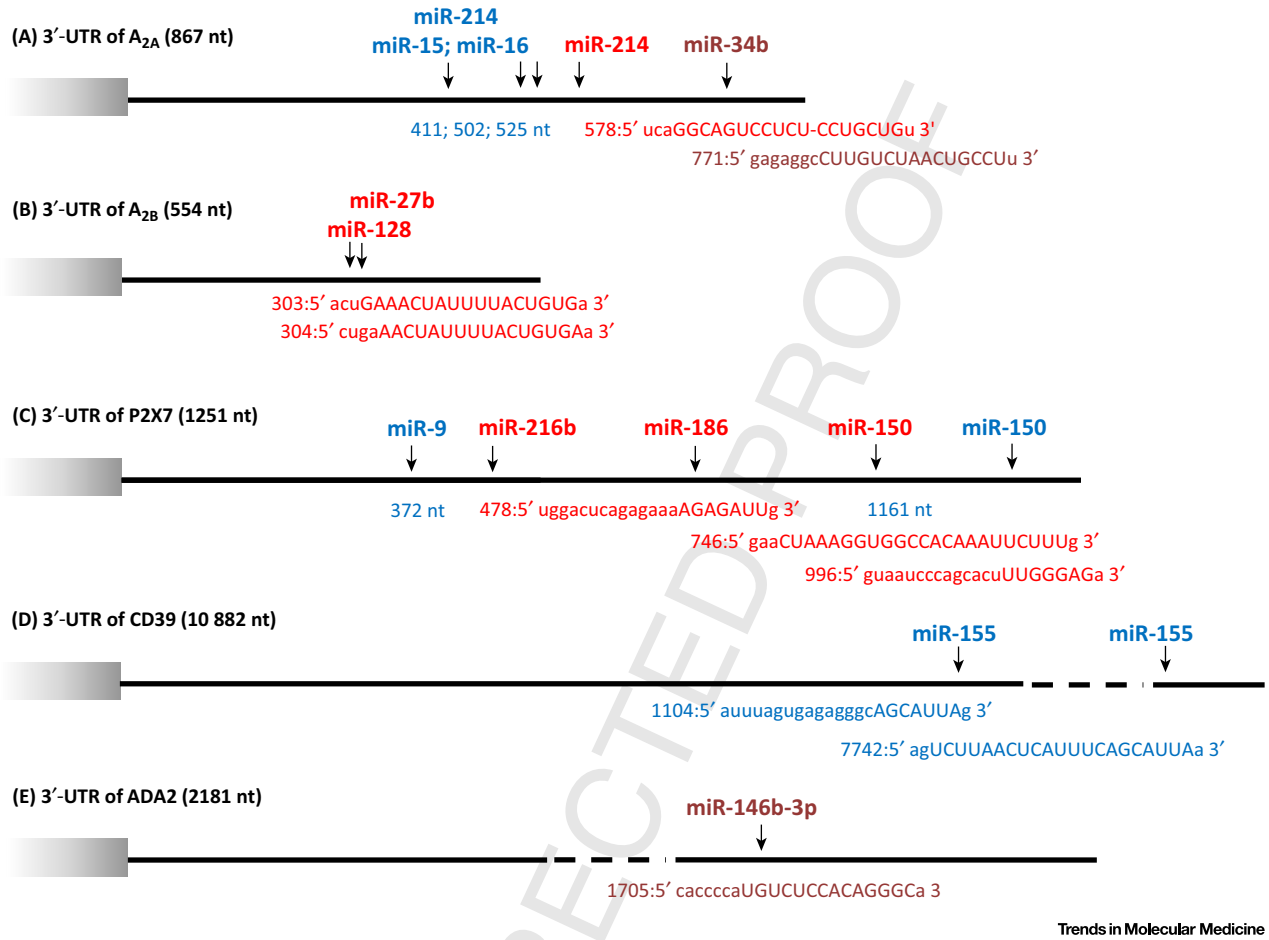


Figure 2. Human 3'-UTR Sequences of Purinergic Receptors and Ectonucleosidases. Represented 3'-untranslated regions (UTRs) are for (A) A_{2A}, (B) A_{2B}, (C) P2X7, (D) CD39, and (E) ADA2. Putative miRNA target sequences were determined using analytical resources available at http://www.microrna.org/. Among all proposed sequences for the A_{2A} receptor, the following have been considered: NM_000675, AK289871, AK301420, AK312946, CR611621, S46950, X68486. For the A_{2B} receptor: NM_000676. For the P2X7 receptor: NM_002562, AY847300, AY847302, AY847304. For CD39: NM_001776, NM_001098175, NM_001164178, NM_001164179. For ADA2: NM_017424, NM_177405, AK304818, AK314321. In red, target sites of conserved miRNAs with good mirSVR scores are shown (algorithm to rank and score miRNA target sites); in blue, target sites of conserved miRNAs are shown with all mirSVR scores; in brown, target sites of all miRNAs with good mirSVR scores are shown.

P1 Receptors Are Modulated by miRNAs

The **A_{2A} receptor** has become an attractive target for medicine and pharmacology owing to its pleiotropic functions, from cancer to inflammatory diseases (human ulcerative colitis, psoriasis, atopic dermatitis, and asthma), neurological diseases (Parkinson, Alzheimer, epilepsy, autism) and cardiovascular diseases [66–69]. Recent data have suggested that the A_{2A} receptor can operate under the control of different miRNAs, including miR-34b, miR-214, miR-15, and miR-16 [70–72]. An interesting study reported that endogenous A_{2A} receptor protein levels were increased when miR-34b was blocked using a specific anti-miR-34b (Table 1) [70]. Moreover, a **luciferase** reporter assay of a mutated miR-34b-predicted binding site within the 3'-untranslated region (UTR) of A_{2A} mRNA abolished the effects of A_{2A} miRNA when a miR-34b mimetic molecule was used. These findings may be relevant in that A_{2A} receptor expression can be elevated in Parkinson's disease patients both in the putamen and in peripheral blood, correlating well with disease severity [73–76].

Table 1. miRNA-mediated Modulation of the Purinergic Network

Purinergic receptors	Regulatory miRNAs	Experimental methods	Refs
A_{2A}	miR-34b	A point mutation in an miR-34b predicted binding site within the 3'UTR region of A_{2A} mRNA abolished the effect of the miRNA using a miR-34b mimetic (luciferase reporter assay). A_{2A} protein levels increased when miR-34b function was blocked by a specific anti-miR-34b.	[70]
A_{2A}	miR-214 miR-15 miR-16	Bioinformatic analyses and reporter gene assays revealed that A_{2A} expression was modulated by miRNA-214, miRNA-15, and miRNA-16.	[71,72]
A_{2A}	miR-16	Colonic mucosa of active ulcerative colitis patients showed overexpression of miR-16 and down-regulation of the A2A receptor. miR-16 negatively regulated A2A expression at the post-transcriptional level.	[66]
A_{2B}	miR-27b miR-128a mir-128b	Binding of miRNAs to the 3'-untranslated region (UTR) of A_{2B} mRNA were demonstrated upon cloning of a 3'-UTR sequence downstream of luciferase (pMIR-REPORT assay).	[84]
P2X7	miR-9	Lentivirus encoding pre-miR-9 was transduced into neurons. CALHM1 short hairpin RNA was subcloned into the pLB vector to generate CALHM1 shRNA-expressing lentiviral vectors. miR-9 was found to regulate P2X7.	[23]
P2X7	miR-150 miR-186	HEK-293 cells heterologously expressing the full-length 3'-UTR-P2X7 luciferase reporter were used. miR-186 and miR-150 inhibitors increased luciferase activity, whereas miR-186 and miR-150 mimics decreased luciferase activity following **act D** treatment.	[95]
P2X7	miR-150	A **microRNA sponge strategy** was used to inhibit miR-150 in *vitro*. The P2X7 3'UTR region bears a highly conserved miR-150-binding motif and its direct interaction with miR-150 was able to down-regulate the P2X7 protein.	[97]
P2X7	miR-216b	Using bioinformatic analyses and 3'UTR luciferase reporter assays, P2X7 mRNA (and protein) could be targeted and downregulated by miR-216b. Down-regulation of miR-216b in breast cancer was inversely associated with P2X7 expression.	[98]
P2X7	miR-22	Analysis of RISC-loaded microRNAs using a high-throughput platform and functional assays suggested that P2X7 was targeted by miR-22. Accordingly, its inhibition increased P2X7 expression and cytokine levels in the hippocampus.	[119]
P2X7	miR-21	Quantification of P2X7 mRNA and mature Let-7 g, miR-21, and miR-205 expression in 96 NSCLC patients using quantitative reverse transcription PCR. Up-regulation of miR-21 corresponded to low P2X7 expression and to a lower survival of non-small cell lung cancer (NSCLC) patients.	[100]
P2X7	miR-22, miR-125b, miR-146b and miR-155	miRNA transcription profile of control mice microglial cells and ALS microglia in resting conditions and upon P2X7 stimulation with the agonist BzATP. miR-22, miR-125b, miR-146b and miR-155 were upregulated upon stimulation. miR-365 and miR-125b interfered with transcription of IL-6 and STAT3 pathways, increasing TNF-α transcription.	[104]
CD39	miR-155	miR-155 was up-regulated in patients with sepsis and in a murine model of sepsis. This was accompanied by an increased number of $CD39^+$ Treg lymphocytes. Their number decreased in mice transfected with a miR-155 inhibitor.	[110]
ADA2	miRNA-146b-3p	miRNA-146b-3p decreased ADA2 expression and *in vitro* TNF-alpha secretion.	[114]

Aside from neurodegenerative disorders, the A_{2A} receptor plays an important role in attenuating inflammatory tissue damage due to ischemic liver injury, lung injury in trauma/hemorrhagic shock, and also progressive kidney fibrosis in A_{2A} KO mice [68]. In this mouse model, reciprocal modulation of miR-214 and A_{2A} has been suggested [71] because both miR-214 and the A_{2A} receptor are involved in inducing fibrotic changes in various organs [77,78]. However, a deeper investigation will be necessary to clarify the specific pathways and effects of downmodulating the expression of these molecules in pathologic states where fibrosis plays a major role.

Of note, it has also been suggested that the A_{2B} receptor subtype is an important regulator of immune responses as well as blood vessel physiology [79]. Recently, miR-15 and miR-16 have been associated with the maturation of NK cells and inhibition of the TGFβ-pathway in mouse heart [80,81]. This will hopefully open the possibility of targeting this subtype to treat immune-mediated pathological states and circulatory dysfunctions. A role for the A_{2B} receptor has also been hypothesized in colitis because this subtype is under the control of TNF-α and is upregulated in mouse models of colitis and human inflammatory bowel disease (IBD). A_{2B} signaling has been suggested to protect animals from colitis [82,83]. At least in the mouse model, A_{2B} mRNA bears four putative miRNA target sites: miR-27a, miR-27b, miR-128a, miR-128b; with miR-27b and miR-128a expression levels being reduced by TNF-α signaling [84]. The relationship between A_{2B} receptor and miRNAs could have important consequences when one considers that this P1 subtype has been implicated in the progression of human oral cancer [85]. In addition, miR-128b has been recently reported to promote apoptosis of malignant cells, suppressing gastric cancer growth by targeting the A_{2B} receptor [86]. Therefore, current evidence for the putative modulation of P1 receptors by miRNAs in different pathologic states justifies further investigative efforts to assess the therapeutic potential of miRNA targeting in purinergic signaling.

P2 Receptors Are Modulated by miRNAs

The purinergic P2X7 receptor subtype is activated by extracellular ATP and mediates a variety of cell responses depending on the cell type, receptor expression, and stimulus duration. Therefore, P2X7 has been a research focus in different pathophysiological conditions, and its activation has been linked to chronic inflammation, diabetes-induced retinal damage, neuronal death and seizures in epilepsy, Alzheimer's disease, cancer and metastasis, as well as in viral infections for both humans and animal models [87–94]. P2X7 receptor expression appears to be regulated by miRNAs. Indeed, different experimental studies (mostly conducted *in vitro* in various cell lines) have identified binding sites within the 3'-UTR region of P2X7 mRNA, and are recognized by different miRNAs, including miR-216b, miR-150, miR-186, miR-22, and miR-9 [95–98]. A role for miR-150 and miR-186 in tumor progression has been reported for different cancers, and expression of these miRNAs has also been linked to inhibition of tumor growth and metastasis [99,100].

In addition, a recent study in human embryonic kidney-293 cells showed that miR-186 and miR-150 inhibitors could increase luciferase activity in a full-length P2X7 3'-UTR luciferase reporter assay [95]. By contrast, miR-186 and miR-150 mimetics decreased luciferase activity following **actinomycin D** treatment (act D) (inhibiting transcription), confirming that miR-150 and miR-186 could downregulate P2X7 through the activation of instability sites at the 3'-UTR of P2X7 mRNA [95]. Moreover, normal epithelial cells have been found to express higher P2X7 mRNA and protein levels relative to pre-cancerous and cancerous human epithelial cells (ectocervical squamous carcinoma, endometrial adenocarcinoma, bladder transitional cell carcinoma, and breast ductal adenocarcinoma) [95]. Similarly, miR-186 and miR-150 are highly expressed in tumors compared to normal cells; in agreement with the proposed regulation of P2X7, treatment of P2X7-expressing neoplastic human cell lines with miR-186 and miR-150 mimetics or inhibitors demonstrated that these two miRNAs can effectively modulate the expression of this purinergic receptor. In particular, upregulation of miRNAs caused a decrease in P2X7 mRNA and this was linked to diminished apoptotic cell death [95].

Anticancer drugs may have an effect on miRNA expression, such as for carboplatin *in vitro*, a chemotherapeutic drug shown to suppress miR-21 and inhibit TGF-β receptor signaling implicated in non-small cell lung cancer (NSCLC) cell invasion in NOD/SCID mice [99]. In NSCLC cells, upregulated miR-21 corresponds to low P2X7 expression and decreased survival of NSCLC patients [100]. Therefore, the recent demonstration of the interplay between P2X7 receptor and miR-21 may be potentially relevant for treating this type of neoplasia. A recent report showed that, although expression of the P2X7 subtype is regulated by miRNAs, activation of the receptor is in turn able to modulate different miRNAs, including miR-21 [101]. In this study, P2X7-mediated activity was linked to the expression of vascular endothelial growth factor (VEGF) and IL-6, and was hypothesized to play a role in human psoriatic skin lesions [101]. Another example linking miRNAs and P2 receptors is based on recent findings on **amyotrophic lateral sclerosis** (ALS), a brain and spinal cord disease where motor neurons progressively degenerate and die. Dysregulation of miRNAs and subsequent neuroinflammation have been suggested as mechanisms involved in ALS pathogenesis [102,103]. Relevant to this issue, the miRNA transcription profile of control murine microglial cells and ALS microglia *ex vivo* has been recently compared under resting and P2X7 receptor stimulation conditions [104]; the potent synthetic agonist 2'/3'-O-(4-benzoylbenzoyl)adenosine-5'-triphosphate (BzATP) induced the upregulation of miR-22, miR-125b, miR-146b, and miR-155 in microglia [104]. Moreover, miR-365 and miR-125b were found to increase the transcription of *TNFA*, encoding a proinflammatory cytokine linked to ALS neuroinflammation [104]. In addition, the involvement of P2 receptors in mediating and maintaining **nociceptive sensitivity**, as well as neuropathic and inflammatory pain, has been ascertained in different studies [93,94,105]. One study linked P2X7 receptor-mediated signaling and its modulation by miR-9 to pain sensation in a rat model of streptozotocin-induced diabetes, opening up another area of research for human pathologic nociception. Diabetic male Sprague–Dawley rats displayed miR-9 and calcium homeostasis modulator 1 (CALHM1) upregulation in spinal dorsal horn neurons [23]. Another study examined miR-22, which has recently emerged as an important regulator of the adhesion protein intercellular adhesion molecule 1 (ICAM-1) that is expressed by endothelial cells and is involved in adhesion and *trans*-endothelial migration of immune cells to tissues. In this study, UTP and ATP were shown to reduce endothelial inflammation via miR-22-induced ICAM-1 inhibition [106]. Stimulation of the immortalized human microvascular endothelial cell line HMEC-1 with extracellular UTP increased the expression of miR-22 and conversely decreased ICAM-1 protein levels. Accordingly, miR-22 overexpression and stimulation with UTP or ATP significantly inhibited leukocyte adhesion [106]. This finding might be potentially relevant for pathologic states where excessive leukocyte adhesion to the endothelium causes damage to endothelial cells (ECs), modifying subendothelial tissue composition, as in atherosclerotic lesions [107].

Are Ectonucleotidases and ADA Modulated by miRNAs?

miR-155 can immunomodulate IL-10-mediated responses in mast cells or **T helper 17 lymphocytes** (Th17) [108–110]. miR-155 has also been shown to stimulate **T regulatory cell** (Treg) and Th17 differentiation upon transfection of $CD4^+$ T cells with pre-miR-155 and anti-miR-155 (110). A recent report showed that miR-155 was upregulated in patients with sepsis and in a murine model of sepsis [109,110]; increased numbers of $CD39^+$ T lymphocytes (Treg) were found in sepsis patients. Although a mechanistic explanation is lacking, the information might be potentially clinically relevant because an elevated number of $CD39^+$ Tregs has been reported to correlate with poor prognosis in sepsis patients [110]. Because miR-155 can be a potent modulator of the immune system, its specific impact on molecular components of the purinergic network might be mediated via a series of complex immunomodulatory processes. Another finding suggesting a link between miR-155 and the purinergic network is the fact that miR-155 deficiency in mouse dendritic cells (DC) has been found to be associated with reduced DC chemotactic responses and defective IL-1β secretion upon ATP-mediated stimulation in a mouse model of allergic airway inflammation [111]. Another report also indicated that miR-155-deficient

DCs could lead to less-severe GVHD in mice through reduced migration and defective inflammasome activation [112].

As discussed, ectonucleotidases CD39 and CD73 play a fundamental role in regulating nucleotide and nucleoside concentrations in the extracellular milieu. Differences in miRNA expression profiles between CD73 immunopositive rods and postnatal Müller glial cells of the mouse retina have been recently described [113], and this may pave the way for future investigations of the human retina. The authors hypothesize that the heterogeneity of miRNAs might contribute to differentiation of retinal precursors. Another relevant point concerns ADA regulation by miRNAs. The enzyme is expressed in two isoforms (ADA1 and ADA2) in humans and is endowed with the ability to degrade the P1 receptor agonist ADO to its derivative inosine, which is inactive at P1 receptors. Degradation of ADO reduces its extracellular concentration, thus halting P1-mediated signaling (Figure 1). Recent data have also suggested that miRNA-146b-3p might be involved in the modulation of retinal inflammation because it is responsible for decreased ADA2 expression and *in vitro* secretion of TNF-α in activated human macrophages [114].

The interplay between miRNAs and nucleotide/nucleoside-metabolizing enzymes suggests that blocking or reducing excessive extracellular agonist concentrations (either ATP or ADO) via miRNA technology could be considered in cases where there is abnormally high activation of particular purinergic receptors, as in the case of retinal inflammation or other chronic inflammatory diseases.

Therapeutic Perspectives

Signaling through purinergic receptors has been the subject of increased interest given the multiplicity of responses elicited by the purinergic network in virtually every tissue. Dysregulation of the purinergic network has been detected in pathologic states ranging from neurodegeneration to allergy; from graft rejection to diabetes, osteoporosis, stroke and cancer; therefore, finding effective and safe ways to transiently or stably block pathologic purinergic signaling activation in a localized manner might be promising means to prevent deleterious consequences for tissues. Indeed, an exciting advance in purinergic signaling biology has been the recent evidence that miRNAs can modulate the expression of molecular components of the purinergic network (Table 1 and Figure 3). This fosters the development of new therapeutic approaches and encourages deciphering the impact of each miRNA on the post-transcriptional regulation of components of the purinergic network. Strategies based on miRNAs activity have been considered in cases of pathologic downregulation of miRNA expression; these include 'miRNA replacement therapy' as well as targeting and inactivating abnormally hyperexpressed miRNAs – in other words, 'therapeutic miRNA targeting'. These approaches have been the subject of great interest with respect to multiple future therapeutic developments, particularly for cancer therapy. Although the number of clinical trials is presently very limited [50,115] and mostly restricted to Phase I studies for the treatment of cancer, notably, one of these, based on miR-16 mimetics (TargomiRs) has been performed in patients failing to respond to standard therapy [MesomiR 1: A Phase I Study of TargomiRs as 2nd or 3rd Line Treatment for Patients with Recurrent Malignant Pleural Mesothelioma (MPM) and NSCLC; www.clinicaltrials.gov]. MRX34, a mir-34a based experimental drug, is also currently under investigation in a Phase I trial in primary liver cancer, SCLC, lymphoma, melanoma, multiple myeloma, renal cell carcinoma, and NSCLC (www.clinicaltrials.gov).

Because miR-16 is also involved in controlling A_{2A} expression, future applications of miRNA technology might be used to decrease A_{2A} receptor expression and ameliorate patient symptoms in pathologic conditions such as in ulcerative colitis [66] and cancer [116,117], or in specific brain regions during the course of Parkinson's disease, but these remain hypothetical. Another promising application of miRNA technology in the treatment of neurological diseases might

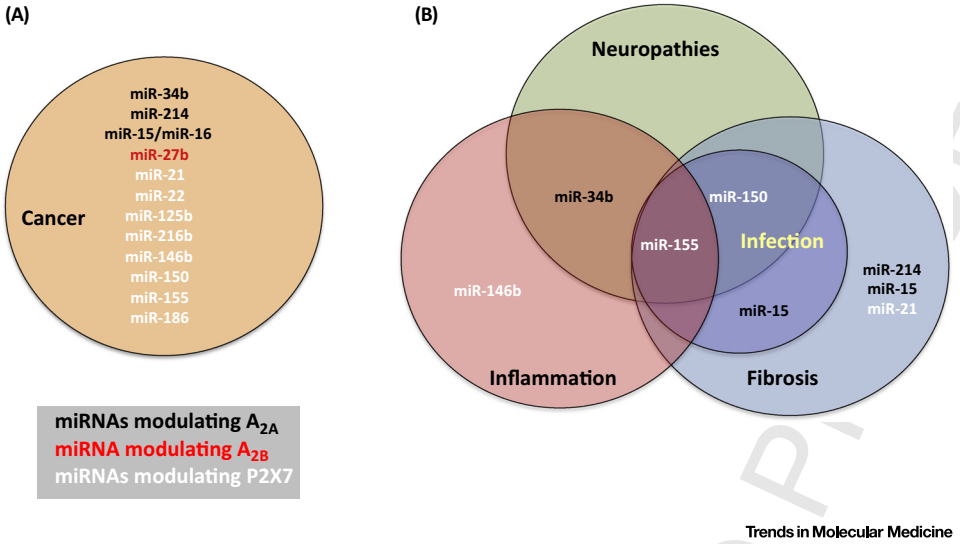


Figure 3. Interplay Between miRNAs in Disease and miRNAs which Modulate Purinergic Receptors. The circles include miRNAs that have been mainly implicated in (A) cancer or (B) in inflammation, neurologic diseases, and fibrotic states. Venn diagram: overlapping regions indicate miRNA intersections (in common) with the reported diseases. miRNAs are indicated in black if modulating the A_{2A} receptor, in red for A_{2B} modulation, and in white for P2X7 modulation.

exploit A_1-mediated neuronal protection, triggering increased expression of this receptor subtype by neuronal cells in neurodegenerative conditions such as Alzheimer's disease [118].

One of the advantages of using miRNA technology to modulate purinergic-mediated responses includes combined targeting of purinergic receptor activation, as in the case of the antagomir214 and the A_{2A} agonist CGS21680, resulting in an overall increased anti-inflammatory response [71]. Encouraging results on the application of miRNA technology have stemmed from experiments performed by *in vivo* injection of miRNA-22 mimetics. Indeed, this approach has been used to transiently suppress spontaneous epilepsy-like seizures in mice [119], suggesting that miRNA-22 might represent a potential therapeutic target for the prevention and/or treatment of inflammatory brain conditions or for the prevention of secondary epileptogenic foci. Similarly, miRNA-related applications might be useful to prevent or mitigate neuronal cell damage and cell death linked to P2X7 receptor activation in ALS [120].

The expression of specific miRNAs or pre-miRNAs has been considered to be important for the evolution and prognosis of several different diseases [121,122], with cancer having a prominent role. To this end, an exciting finding in tumor biology has been the demonstration that upregulation of miR-150 is inversely correlated with P2X7 expression, thus promoting cancer growth in human endometrial and ectocervical tissues [95]. Different studies have shown that high levels of miR-21 are associated with the downregulation of tumor-suppressor genes, including the *Ras* homolog gene family member B, programmed cell death protein 4 (*PDCD4*), tissue inhibitor of metalloproteinase 3, phosphatase and tensin homolog (*PTEN*), and tropomyosin 1. Accordingly, miR-21 silencing causes cell-cycle arrest and increased chemosensitivity to anticancer drugs, as demonstrated for human gastric cancer. Given the relevance of both miR-21 and P2X7 to tumor biology, the interplay between these should be examined in detail. Another point deserving thorough investigation concerns the regulation of miRNA expression by purinergic receptors. The stimulation of cells with specific P1 or P2 agonists remains a largely unexplored and potentially useful means by which to modulate the expression of different target miRNA families.

Box 1. Clinician's Corner

Purinergic receptors modulate many biological functions including heart rate, vascular tone, neuronal excitation, tissue repair, and immune responses in humans.

Alteration of the purinergic signaling network is associated with a variety of human diseases, such as rheumatoid arthritis, Alzheimer's disease, multiple sclerosis, and epilepsy; a consistent number of these diseases have no real cure and await therapies that might involve normalizing purinergic signaling dysregulation and thereby ameliorating patient symptoms. miRNAs are key regulators of gene expression, gaining attention as novel disease biomarkers and potential therapeutic targets. Recent studies have shown that purinergic cell-surface receptors activated by extracellular nucleotides and nucleosides are subject to miRNA regulation.

Healthcare providers interested in the management of patients affected by diseases associated with alterations in purinergic signaling may benefit from an increased understanding of miRNA-based regulation of the purinergic signaling network. Indeed, miRNA regulation has been proposed as a potential therapeutic strategy in cases where miRNA activity should be inhibited (antagomiRNA therapeutics), or mimicked (miRNA replacement therapy). Some of these approaches are currently undergoing clinical trials [Phase I study on the effects of a miRNA replacement strategy based on miR-16 mimetic (TargomiRs) in recurrent malignant pleural mesothelioma (MPM)].

Presently, studies on miRNAs regulation of purinergic signaling are mainly at proof-of-concept stage where it is presumed that miRNA targeting might be useful to correct purinergic signaling dysregulation. Hypothetically, novel therapeutic strategies specifically targeting miRNA/mRNA networks could be established. This might potentially be achieved by co-manipulating the expression of miRNA sets and introducing novel delivery strategies. Combination drug treatments and miRNA-based approaches are also conceivable.

Outstanding Questions

Is it possible to target proinflammatory molecular components of the purinergic network to treat inflammatory diseases in humans?

Could miRNAs implicated in the control of P2X7 receptor expression be useful in blocking tumor progression?

Are P2Y receptors under the control of miRNAs? How does this affect cell-specific function and differentiation of different cell types?

Do molecular variants of purinergic receptors present different or redundant miRNA regulatory pathways? Could they induce different sets of miRNAs and could this impact on disease states?

Moreover, therapies based on the modulation of specific miRNAs in conditions where dysregulation of purinergic signaling has been implicated could hopefully be introduced to treat acute or chronic conditions including sepsis, colitis, and allergies, as well as cancers and neurological diseases [82,89,90].

Concluding Remarks and Future Directions

Although methodological advances in the proposed approaches for synthetic miRNA or anti-miRNA delivery are warranted, miRNA-mediated modulation of the purinergic signaling network may provide a concrete chance to treat many human diseases in the not-so-distant future (Box 1 and Outstanding Questions). Such strategies might be expected to be more specific than commonly used pharmaceuticals, accurately targeting molecular networks. Moreover, combined treatments using pre-miRNA and anti-miRNA molecules represent, at least in theory, a valuable tool for targeting not only single miRNAs but also multiple miRNAs involved in different regulatory networks. In the latter case, low concentrations of miRNA-targeting therapeutic molecules (either antagomiRNA or miRNA mimetics) might succeed in preventing unwanted non-specific effects, while maintaining high selectivity and efficacy towards clinically relevant targets. miRNA masking is one such strategy, where instead of targeting an entire miRNA molecule (expected to regulate multiple mRNA targets) fully matching molecular hybridization of an miRNA binding site within the miRNA-regulated mRNA sequence can be performed. In this case, 'masking molecules' for specific mRNAs may be able to inhibit miRNA/mRNA interactions that fall short of the required length and full complementarity to the target site, thereby minimizing non-specific effects. Many avenues are to be followed in these clinically relevant areas of health and disease, but the future may indeed hold exciting and promising answers.

Q4 **Uncited references**

[123–125]

Q5 **Acknowledgments**

The authors apologize to the many authors whose excellent work they could not cite owing to space limitation. The work was supported by National Institutes of Health grants R01 DK097075, R01-HL092188, R01- HL098294, POI-HL114457, Q6 and R01-HL119837 to H.K.E. R.G. was supported by CIB, by COFIN-2009, and by the Associazione Italiana per la Ricerca

sul Cancro (AIRC; IG 13575 – Peptide nucleic acids targeting oncomiR and tumor-suppressor miRNAs: cancer diagnosis and therapy). D.F. was supported by local funds of the University of Ferrara.

References

1. Khakh, B.S. and Burnstock, G. (2009) The double life of ATP. *Sci. Am.* 301, 84–90
2. Fredholm, B. and Verkhratsky, A. (2010) Purines – 80 years and very much alive. *Acta Physiol.* 199, 91–92
3. Burnstock, G. (2007) Purine and pyrimidine receptors. *Cell Mol. Life Sci.* 64, 1471–1483
4. Abbracchio, M.P. *et al.* (2006) International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol. Rev.* 58, 281–341
5. Harden, T.K. *et al.* (2010) Signalling and pharmacological properties of the P2Y receptor. *Acta Physiol.* 199, 149–160
6. Surprenant, A. and North, A. (2009) Signaling at purinergic P2X receptors. *Annu. Rev. Physiol.* 71, 333–359
7. Fredholm, B.B. *et al.* (2011) International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors – an update. *Pharmacol. Rev.* 63, 1–34
8. Zimmermann, H. *et al.* (2012) Cellular function and molecular structure of ecto-nucleotidases. *Purinergic Signal.* 8, 437–502
9. Yegutkin, G.G. (2008) Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade. *Biochim. Biophys. Acta* 178, 673–694
10. Burnstock, G. and Pelleg, A. (2015) Cardiac purinergic signalling in health and disease. *Purinergic Signal.* 11, 1–46
11. Burnstock, G. and Ralevic, V. (2013) Purinergic signaling and blood vessels in health and disease. *Pharmacol. Rev. Dec.* 66, 102–192
12. Haskó, G. and Cronstein, B. (2013) Regulation of inflammation by adenosine. *Front. Immunol.* 4, 85–93
13. Idzko, M. *et al.* (2014) Extracellular nucleotide and nucleoside signaling in vascular and blood disease. *Blood* 124, 1029–1037
14. Ferrari, D. *et al.* (2016) Purinergic signaling in scarring. *FASEB J.* 30, 3–12
15. Lazarowski, E.R. (2012) Vesicular and conductive mechanisms of nucleotide release. *Purinergic Signal.* 8, 359–373
16. Sawada, K. *et al.* (2008) Identification of a vesicular nucleotide transporter. *Proc. Natl. Acad. Sci. U.S.A.* 105, 5683–5686
17. Lohman, A.W. *et al.* (2012) Mechanisms of ATP release and signalling in the blood vessel wall. *Cardiovasc. Res.* 95, 269–280
18. Idzko, M. *et al.* (2007) Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat. Med.* 13, 913–919
19. Kurashima, Y. *et al.* (2012) Extracellular ATP mediates mast cell-dependent intestinal inflammation through P2X7 purinoceptors. *Nat. Commun.* 3, 1034–1046
20. Müller, T. *et al.* (2011) A potential role for P2X7R in allergic airway inflammation in mice and humans. *Am. J. Respir. Cell Mol. Biol.* 44, 456–464
21. Ulmann, L. *et al.* (2010) P2X4 receptors mediate PGE2 release by tissue-resident macrophages and initiate inflammatory pain. *EMBO J.* 29, 2290–2300
22. Parvathenani, L.K. *et al.* (2003) P2X7 mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer's disease. *J. Biol. Chem.* 278, 13309–13317
23. Ferrari, D. *et al.* (2015) Purinergic signaling in atherosclerosis. *Trends Mol. Med.* 21, 184–192
24. Baljit, S. *et al.* (2012) Neuromodulation by extracellular ATP and P2X receptors in the CNS. *Neuron* 76, 51–69
25. Aymeric, L. *et al.* (2010) Tumor cell death and ATP release prime dendritic cells and efficient anticancer immunity. *Cancer Res.* 70, 855–888
26. Schneider, G. *et al.* (2015) Extracellular nucleotides as novel, underappreciated pro-metastatic factors that stimulate purinergic signaling in human lung cancer cells. *Mol. Cancer* 14, 201–216
27. Marino, M. *et al.* (2014) Cangrelor: review of the drug and the CHAMPION programme (including PHOENIX). *Curr. Cardiol. Rep.* 16, 493
28. Liu, F. *et al.* (2015) P2Y12 receptor inhibitors for secondary prevention of ischemic stroke. *Expert. Opin. Pharmacother.* 16, 1149–1165
29. North, R.A. and Jarvis, M.F. (2013) P2X receptors as drug targets. *Mol. Pharmacol.* 83, 759–769
30. He, L. and Hannon, G.J. (2004) MicroRNAs: small RNAs with a big role in gene regulation. *Nat. Rev. Genet.* 5, 522–531
31. Krol, J. and Krzyzosiak, W.J. (2006) Structure analysis of microRNA precursors. *Methods Mol. Biol.* 342, 19–32
32. Krol, J. *et al.* (2007) Ribonuclease dicer cleaves triplet repeat hairpins into shorter repeats that silence specific targets. *Mol. Cell.* 25, 575–586
33. Albertini, M.C. *et al.* (2011) Predicting microRNA modulation in human prostate cancer using a simple String IDentifier (SID1.0). *J. Biomed. Inform.* 44, 615–620
34. Kozomara, A. and Griffiths-Jones, S. (2014) miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res.* 42, D68–D73
35. Alvarez-Garcia, I. and Miska, E.A. (2005) MicroRNA functions in animal development and human disease. *Development* 132, 4653–4662
36. Sontheimer, E.J. and Carthew, R.W. (2005) Silence from within: endogenous siRNAs and miRNAs. *Cell* 122, 9–12
37. Zhang, Z. *et al.* (2011) Uracils at nucleotide position 9-11 are required for the rapid turnover of miR-29 family. *Nucleic Acids Res.* 39, 4387–4395
38. Taccioli, C. *et al.* (2009) UCbase & miRfunc: a database of ultraconserved sequences and microRNA function. *Nucleic Acids Res.* 37, D41–D48
39. Thor, T. *et al.* (2015) MiR-34a deficiency accelerates medulloblastoma formation in vivo. *Int. J. Cancer* 136, 2293–2303
40. Kumar, M.S. *et al.* (2008) Suppression of non-small cell lung tumor development by the let-7 microRNA family. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3903–3908
41. van der Ree, M.H. *et al.* (2016) Miravirsen dosing in chronic hepatitis C patients results in decreased microRNA-122 levels without affecting other microRNAs in plasma. *Aliment. Pharmacol. Ther.* 43, 102–113
42. Gaughwin, P.M. *et al.* (2011) Hsa-miR-34b is a plasma-stable microRNA that is elevated in pre-manifest Huntington's disease. *Hum. Mol. Genet.* 20, 2225–2237
43. Aziz, F. (2016) The emerging role of miR-223 as novel potential diagnostic and therapeutic target for inflammatory disorders. *Cell Immunol.* 303, 1–6
44. Byun, H.M. *et al.* (2004) Erythrocyte ghost-mediated gene delivery for prolonged and blood-targeted expression. *Gene. Ther.* 11, 492–496
45. Wu, Y. *et al.* (2011) MicroRNA delivery by cationic lipoplexes for lung cancer therapy. *Mol. Pharm.* 8, 1381–1389
46. Trang, P. *et al.* (2011) Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. *Mol. Ther.* 19, 1116–1122
47. Babar, I.A. *et al.* (2012) Nanoparticle-based therapy in an in vivo microRNA-155 (miR-155)-dependent mouse model of lymphoma. *Proc. Natl. Acad. Sci. U.S.A.* 109, E1695–E1704
48. Pan, Y. *et al.* (2012) Development of a microRNA delivery system based on bacteriophage MS2 virus-like particles. *FEBS J.* 279, 1198–1208
49. Ohno, S. *et al.* (2013) Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. *Mol. Ther.* 21, 185–191

50. Janssen, H.L.A. *et al.* (2013) Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med.* 368, 1685–1694
51. Lambertucci, C. *et al.* (2015) Medicinal chemistry of P2X receptors: agonists and orthosteric antagonists. *Curr. Med. Chem.* 22, 915–928
52. von Kügelgen, I. and Hoffmann, K. (2016) Pharmacology and structure of P2Y receptors. *Neuropharmacology* 104, 50–61
53. Leone, R.D. *et al.* (2015) A2aR antagonists: next generation checkpoint blockade for cancer immunotherapy. *Comput. Struct. Biotechnol. J.* 13, 265–272
54. Faria, R. *et al.* (2012) Action of natural products on p2 receptors: a reinvented era for drug discovery. *Molecules* 17, 13009–130025
55. Hashemzadeh, M. *et al.* (2009) ADP receptor-blocker thienopyridines: chemical structures, mode of action and clinical use. *J. Invasive Cardiol.* 21, 406–412
56. Cattaneo, M. (2009) New P2Y12 blockers. *J. Thromb. Haemost.* 7, 262–265
57. Mulryan, K. *et al.* (2000) Reduced vas deferens contraction and male infertility in mice lacking P2X1 receptors. *Nature* 403, 86–89
58. Vessey, K.A. and Fletcher, E.L. (2012) Rod and cone pathway signalling is altered in the P2X7 receptor knock out mouse. *PLoS ONE* 7, e29990
59. Webster, C.M. *et al.* (2013) Microglial P2Y12 deficiency/inhibition protects against brain ischemia. *PLoS ONE* 8, e70927
60. Li, D. *et al.* (2012) Roles of purinergic receptor P2Y, G protein-coupled 12 in the development of atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 32, e81–e89
61. Wang, L. *et al.* (2013) Graft-versus-host disease is enhanced by selective CD73 blockade in mice. *PLoS ONE* 8, e58397
62. Peterson, J.R. *et al.* (2014) Treatment of heterotopic ossification through remote ATP hydrolysis. *Sci. Transl. Med.* 6, 255ra132
63. Moeckel, D. *et al.* (2014) Optimizing human apyrase to treat arterial thrombosis and limit reperfusion injury without increasing bleeding risk. *Sci. Transl. Med.* 6, 248ra105
64. Ibrahim, M. *et al.* (2015) Human recombinant apyrase therapy protects against canine pulmonary ischemia-reperfusion injury. *J. Heart Lung Transplant.* 34, 247–253
65. Chen, Y. *et al.* (2015) In vivo delivery of miRNAs for cancer therapy: challenges and strategies. *Adv. Drug Deliv. Rev.* 81, 128–141
66. Tian, T. *et al.* (2016) MicroRNA-16 is putatively involved in the NF-κB pathway regulation in ulcerative colitis through adenosine A2a receptor (A2aAR) mRNA targeting. *Sci. Rep.* Aug 1
67. Le, D.E. *et al.* (2012) Detection of coronary stenosis with myocardial contrast echocardiography using regadenoson, a selective adenosine A2A receptor agonist. *Eur. Heart J. Cardiovasc. Imaging* 13, 298–308
68. Day, Y.J. *et al.* (2004) Protection from ischemic liver injury by activation of A2A adenosine receptors during reperfusion: inhibition of chemokine induction. *Am. J. Physiol. Gastrointest. Liver Physiol.* 286, G285–G293
69. Yuan, G. *et al.* (2015) Novel approaches for targeting the adenosine A2A receptor. *Expert. Opin. Drug Discov.* 10, 63–80
70. Villar-Menéndez, I. *et al.* (2014) Increased striatal adenosine A2A receptor levels is an early event in Parkinson's disease-related pathology and it is potentially regulated by miR-34b. *Neurobiol. Dis.* 69, 206–214
71. Zhao, L. *et al.* (2015) The mutual regulation between miR-214 and A2AR signaling plays an important role in inflammatory response. 27, 2026–2034 Q7
72. Heyn, J. *et al.* (2012) Adenosine A2A receptor upregulation in human PMNs is controlled by miRNA-214, miRNA-15, and miRNA-16. *Shock* 37, 156–163
73. Calon, F. *et al.* (2004) Increased adenosine A2A receptors in the brain of Parkinson's disease patients with dyskinesias. *Brain* 127, 1075–1084
74. Casetta, I. *et al.* (2014) A(2A) adenosine receptors and Parkinson's disease severity. *Acta Neurol. Scand.* 129, 276–281
75. Varani, K. *et al.* (2010) A2A adenosine receptor overexpression and functionality, as well as TNF-alpha levels, correlate with motor symptoms in Parkinson's disease. *FASEB J.* 24, 587–598
76. Vallelunga, A. *et al.* (2014) Identification of circulating microRNAs for the differential diagnosis of Parkinson's disease and Multiple System Atrophy. *Front. Cell Neurosci.* 8, 156–166
77. Denby, L. *et al.* (2014) MicroRNA-214 antagonism protects against renal fibrosis. *J. Am. Soc. Nephrol.* 25, 65–80
78. Perez-Aso, M. *et al.* (2014) Adenosine 2A receptor promotes collagen production by human fibroblasts via pathways involving cyclic AMP and AKT but independent of Smad2/3. *FASEB J.* 28, 802–812
79. Yang, D. *et al.* (2006) The A2B adenosine receptor protects against inflammation and excessive vascular adhesion. *J. Clin. Invest.* 116, 1913–1923
80. Sullivan, R.P. *et al.* (2015) MicroRNA-15/16 antagonizes Myb to control NK cell maturation. *J. Immunol.* 195, 2806–2817
81. Tijsen, A.J. *et al.* (2014) The microRNA-15 family inhibits the TGFβ-pathway in the heart. *Cardiovasc. Res.* 104, 61–71
82. Kolachala, V.L. *et al.* (2005) TNF-alpha upregulates adenosine 2b (A2b) receptor expression and signaling in intestinal epithelial cells: a basis for A2bR overexpression in colitis. *Cell Mol. Life Sci.* 62, 2647–2657
83. Kolachala, V.L. *et al.* (2008) A2B adenosine receptor gene deletion attenuates murine colitis. *Gastroenterology* 135, 861–870
84. Kolachala, V.L. *et al.* (2010) Adenosine 2B receptor expression is post-transcriptionally regulated by microRNA. *J. Biol. Chem.* 285, 18184–18190
85. Kasama, H. *et al.* (2015) Adenosine A2b receptor promotes progression of human oral cancer. *BMC Cancer* 15, 563–575
86. Wang, P. *et al.* (2015) MicroRNA-128b suppresses tumor growth and promotes apoptosis by targeting A2bR in gastric cancer. *Biochem. Biophys. Res. Commun.* 467, 798–804
87. Baudelet, D. *et al.* (2015) Involvement of the P2X7 purinergic receptor in inflammation: an update of antagonists series since 2009 and their promising therapeutic potential. *Curr. Med. Chem.* 22, 713–729
88. Giannuzzo, A. *et al.* (2015) The P2X7 receptor regulates cell survival, migration and invasion of pancreatic ductal adenocarcinoma cells. *Mol. Cancer* 14, 203–218
89. Sugiyama, T. (2014) Role of P2X7 receptors in the development of diabetic retinopathy. *World J. Diabetes* 5, 141–145
90. Sperlágh, B. and Illes, P. (2014) P2X7 receptor: an emerging target in central nervous system diseases. *Trends Pharmacol. Sci.* 35, 537–547
91. Swartz, T.H. *et al.* (2015) Purinergic receptors: key mediators of HIV-1 infection and inflammation. *Front. Immunol.* 6, 585–594
92. Volonté, C. *et al.* (2012) Purinergic signalling: what is missing and needed next? The use of transgenic mice, crystallographic analysis and microRNA. *CNS Neurol. Disord. Drug Targets* 11, 751–767
93. Kobayashi, K. *et al.* (2011) Induction of the P2X7 receptor in spinal microglia in a neuropathic pain model. *Neurosci. Lett.* 504, 57–61
94. Bele, T. and Fabbretti, E. (2015) P2X receptors, sensory neurons and pain. *Curr. Med. Chem.* 22, 845–850
95. Zhou, L. *et al.* (2008) MicroRNAs miR-186 and miR-150 down-regulate expression of the pro-apoptotic purinergic P2X7 receptor by activation of instability sites at the 3′-untranslated region of the gene that decrease steady-state levels of the transcript. *J. Biol. Chem.* 283, 28274–32886
96. Rahman, O.A. *et al.* (2010) Analysis of a polymorphic microRNA target site in the purinergic receptor P2RX7 gene. *Electrophoresis* 31, 1790–1795
97. Huang, S. *et al.* (2013) miR-150 promotes human breast cancer growth and malignant behavior by targeting the pro-apoptotic purinergic P2X7 receptor. *PLoS ONE* 8, e80707
98. Zheng, L. *et al.* (2014) Regulation of the P2X7R by microRNA-216b in human breast cancer. *Biochem. Biophys. Res. Commun.* 452, 197–204

99. Lin, L. *et al.* (2016) MicroRNA-21 regulates non-small cell lung cancer cell invasion and chemo-sensitivity through SMAD7. *Cell Physiol. Biochem.* 38, 2152–2162

100. Boldrini, L. *et al.* (2015) P2X7 mRNA expression in non-small cell lung cancer: MicroRNA regulation and prognostic value. *Oncol. Lett.* 9, 449–453

101. Killeen, M.E. *et al.* (2013) Signaling through purinergic receptors for ATP induces human cutaneous innate and adaptive Th17 responses: implications in the pathogenesis of psoriasis. *J. Immunol.* 190, 4324–4336

102. Phani, S. *et al.* (2012) The role of the innate immune system in ALS. *Front. Pharmacol.* 3, 150–156

103. Ehrhart, J. *et al.* (2015) Humoral factors in ALS patients during disease progression. *J. Neuroinflammation* 12, 127–138 Q9

104. Parisi, C. *et al.* (2016) MicroRNA-125b regulates microglia activation and motor neuron death in ALS. *Cell Death Differ.* 23, 531–541

105. Burnstock, G. (2016) Purinergic mechanisms and pain. *Adv. Pharmacol.* 75, 91–137

106. Gidlöf, O. *et al.* (2015) Extracellular uridine triphosphate and adenosine triphosphate attenuate endothelial inflammation through miR-22-mediated ICAM-1 inhibition. *J. Vasc. Res.* 52, 71–80

107. Liu, W. *et al.* (2016) miR-9 mediates CALHM1-activated ATP–P2X7R signal in painful diabetic neuropathy rats. *Mol. Neurobiol.* Published online January 19, 2016. http://dx.doi.org/10.1007/s12035-016-9700-1 Q8

108. Qayum, A.A. *et al.* (2016) IL-10-induced miR-155 targets SOCS1 to enhance IgE-mediated mast cell function. *J. Immunol.* 196, 4457–4467 Q10

109. Yao, R. *et al.* (2012) MicroRNA-155 modulates Treg and Th17 cells differentiation and Th17 cell function by targeting SOCS1. *PLoS ONE* 7, e46082 Q11

110. Liu, J. *et al.* (2015) Elevated miR-155 expression induces immunosuppression via CD39$^+$ regulatory T-cells in sepsis patient. *Int. J. Infect. Dis.* 40, 135–141 Q12

111. Zech, A. *et al.* (2015) MicroRNA-155 modulates P2R signaling and Th2 priming of dendritic cells during allergic airway inflammation in mice. *Allergy* 70, 1121–1129 Q13

112. Chen, S. *et al.* (2015) MicroRNA-155-deficient dendritic cells cause less severe GVHD through reduced migration and defective inflammasome activation. *Blood* 126, 103–112

113. Quintero, H. *et al.* (2016) MicroRNA changes through Müller glia dedifferentiation and early/late rod photoreceptor differentiation. *Neuroscience* 16, 109–121

114. Fulzele, S. *et al.* (2015) MicroRNA-146b-3p regulates retinal inflammation by suppressing adenosine deaminase-2 in diabetes. *Biomed. Res. Int.* 846501

115. Bouchie, A. (2013) First microRNA mimic enters clinic. *Nat. Biotech.* 31, 577

116. Kang, W. (2015) Targeting of YAP1 by microRNA-15a and microRNA-16-1 exerts tumor suppressor function in gastric adenocarcinoma. *Mol. Cancer* 14, 52–62

117. Venturutti, L. *et al.* (2016) MiR-16 mediates trastuzumab and lapatinib response in ErbB-2-positive breast and gastric cancer via its novel targets CCNJ and FUBP1. *Oncogene* Published online May 9, 2016. http://dx.doi.org/10.1038/onc.2016.151

118. Nhan, H.S. *et al.* (2015) The multifaceted nature of amyloid precursor protein and its proteolytic fragments: friends and foes. *Acta Neuropathol.* 129, 1–19

119. Jimenez-Mateos, E.M. *et al.* (2015) microRNA targeting of the P2X7 purinoceptor opposes a contralateral epileptogenic focus in the hippocampus. *Sci. Rep.* 5, 17486–17503

120. Apolloni, S. *et al.* (2013) The NADPH oxidase pathway is dysregulated by the P2X7 receptor in the SOD1-G93A microglia model of amyotrophic lateral sclerosis. *J. Immunol.* 190, 5187–5195

121. Zhang, Z. *et al.* (2016) Genetic variants in microRNAs predict recurrence of ischemic stroke. *Mol. Neurobiol.* Published online March 24, 2016. http://dx.doi.org/10.1007/s12035-016-9865-7

122. Gimondi, S. *et al.* (2016) A circulating miRNA panel for prediction of acute graft-versus-host disease in lymphoma patients undergoing matched unrelated hematopoietic stem cell transplantation. *Exp. Hematol.* 44, 624–634

123. Bertoli, G. *et al.* (2016) MicroRNAs as biomarkers for diagnosis, prognosis and theranostics in prostate cancer. *Int. J. Mol. Sci.* 17, 421

124. Sekar, D. *et al.* (2016) Significance of microRNA 21 in gastric cancer. *Clin. Res. Hepatol. Gastroenterol.* Published online May 11, 2016. http://dx.doi.org/10.1016/j.clinre.2016.02.010

125. Wang, Q. *et al.* (2004) P2X7 receptor-mediated apoptosis of human cervical epithelial cells. *Am. J. Physiol. Cell Physiol.* 287, C1349-1358