Review

JHEP|Reports

Purinergic signalling in liver diseases: Pathological functions and therapeutic opportunities

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Summary

Extracellular nucleotides, including ATP, are essential regulators of liver function and serve as danger signals that trigger inflammation upon injury. Ectonucleotidases, which are expressed by liver-resident cells and recruited immune cells sequentially hydrolyse nucleotides to adenosine. The nucleotide/nucleoside balance orchestrates liver homeostasis, tissue repair, and functional restoration by regulating the crosstalk between liver-resident cells and recruited immune cells. In this review, we discuss our current knowledge on the role of purinergic signals in liver homeostasis, restriction of inflammation, stimulation of liver regeneration, modulation of fibrogenesis, and regulation of carcinogenesis. Moreover, we discuss potential targeted therapeutic strategies for liver diseases based on purinergic signals involving blockade of nucleotide receptors, enhancement of ectonucleoside triphosphate diphosphohydrolase activity, and activation of adenosine receptors.

Keywords: Purinergic signals; Liver; Adenosine receptors; Nucleotide receptors; Ectonucleoside triphosphate diphosphohydrolases 1; Ecto-5′-nucleotidase

Received 28 April 2020; received in revised form 24 June 2020; accepted 22 July 2020Available online 30 July 2020

Introduction

Purines and purine nucleotides are the oldest multifunctional biological molecules in evolutionary history. In addition to acting as universal intracellular energy currencies for biological reactions, ATP and its hydrolytic products, including ADP, AMP, and adenosine, serve as essential extracellular signals involved in physiological processes and pathological conditions.[1,2] The purinergic signalling system consists of 3 major steps: release of intracellular nucleotides into the extracellular space, activation of type 1 (P1) and type 2 (P2) purinergic receptor families with autocrine and paracrine effects, and regulation of relative extracellular nucleotide/adenosine levels by ectonucleoside triphosphate diphosphohydrolases (NTPDases) and ecto-5′-nucleotidase (CD73) to terminate nucleotide signals (Fig. 1).[3]

Over the past 5 years, several reviews have extensively highlighted that purinergic signals regulate liver function and injury responses.[4–7] Moreover, the roles of purinergic signals in liver inflammation and fibrosis have recently been summarised.[8,9] In the current review, we focus on recent evidence supporting the effects of purinergic signals on liver function and homeostasis, restriction of inflammation, stimulation of liver regeneration, modulation of fibrogenesis, and regulation of carcinogenesis. Furthermore, we discuss the potential therapeutic applications of adapting purinergic signalling for liver diseases of varying aetiology.

Functions of purine signalling in liver physiology and pathology

Liver-resident cells (Table 1) and immune cells recruited upon injury (Table 2) express their own purinoceptor subtypes and ectonucleotidases that respond to and regulate extracellular purinergic signals (Fig. 2). Two families of purinoceptors have been identified: purinergic 1 (P1) and purinergic 2 (P2) receptors. P1 receptors are activated by adenosine and include 4 receptor types: adenosine receptor A (Adora)-1 (A1), Adora- 2A (A2A), Adora-2B (A2B), and Adora-3 (A3). P2 receptors are divided into 2 subgroups, with 1 subgroup consisting of 7 subtypes of ATP-gated channel P2X receptors ($P2X_{1-7}$), which only bind to ATP, and the other subgroup consisting of 8 subtypes of Ca^{2+}/phosphatidylinositol-coupled P2Y receptors ($P2Y_{1,2,4,6,11-14}$), which can be activated by both ATP and other nucleotides, including ADP for $P2Y_{1,11,13}$, nicotinic acid adenine dinucleotide phosphate for $P2Y_{11}$, uridine triphosphate (UTP) for $P2Y_{2,4,6,11}$, uridine diphosphate (UDP) for $P2Y_6$, and UDP-glucose for $P2Y_{14}$, thus serving as agonists of these P2Y receptors.[3]

The NTPDase family is composed of 8 proteins (NTPDases 1–8), 4 of which (NTPDases 1, 2, 3 and 8) face the extracellular space. In the liver, NTPDase1 (also called CD39) is mainly expressed by endothelial cells and recruited immune cells, wherein it hydrolyses ATP and ADP to AMP; NTPDase2 is expressed by activated hepatic stellate cells (HSCs) and myofibroblasts and only

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hydrolyses ATP to ADP; NTPDase8 is restricted to the bile canaliculi of hepatocytes and ATP is its preferred substrate. CD39 is the rate-limiting enzyme for generation of AMP, which is ultimately converted into adenosine by CD73.

Under physiological conditions, nanomolar levels of ATP in the extracellular space maintain liver function and homeostasis (Fig. 1). Upon liver injury, millimolar levels of ATP can be detected in the extracellular space, as a consequence of tissue damage-related cell stress or cell death. After hydrolysis of ATP by NTPDases and CD73, the extracellular adenosine concentration increases from the nanomolar range (100 to 500 nM) to the micromolar range under physiological conditions, in response to inflammation, hypoxia, and ischaemia (Fig. 1). During the reconstitution process in the liver, purinergic signals exert their effects on liver-resident cells and recruit immune cells to restrict inflammation, stimulate regeneration, modulate fibrogenesis, and regulate carcinogenesis (Fig. 3).

Key points

- Purinergic signalling plays an essential role in liver function and homeostasis by mediating hepatocyte-cholangiocyte communication.
- Injury-induced release of ATP into the extracellular area serves as a danger alert signal for immune cell recruitment, and CD39 and CD73 expressed by the recruited cells scavenge extracellular ATP to generate immunosuppressive adenosine.
- Purinergic signalling stimulates liver regeneration by modulating the interaction of hepatocytes, sinusoidal endothelial cells, natural killer cells, and recruited haematopoietic stem cells.
- Nucleotide receptors exert different effects on biliary and non-biliary fibrosis, and adenosine and its receptors have discrepant effects on liver fibrosis *in vivo*.
- Nucleotides induce hepatocyte maltransformation and stimulate hepatocellular carcinoma cell proliferation and metastasis, while the effects of adenosine on carcinogenesis are still conflicting.
- Based on purinergic signals, blockade of nucleotide receptors, enhancement of NTPDase activity, and activation of adenosine receptors have shown promising therapeutic effects in liver diseases.

Homeostasis maintenance

Under physiological conditions, hepatocytes and cholangiocytes continuously release ATP into the bile at a concentration of approximately 5 μM in humans for functional preservation and hepatocyte-cholangiocyte communication.[10] Extracellular ATP controls multiple essential functions of hepatocytes, including glucose metabolism, cholesterol transport, and bile secretion, which are mediated by functional $P2X_4$ and $P2Y_{1,2,4,6,13}$ receptors on the basolateral and canalicular domains (Fig. 2).[11–16] In cholangiocytes, ATP is involved in bile secretion through Cl^- channel activation and Na^+/H^+ exchange.[17,18] As the hydrolytic product of ATP, adenosine activates the P1 receptors on hepatocytes to mediate Ca^{2+}-dependent or cAMP-dependent ureagenesis, glycogenolysis, and gluconeogenesis.[19,20] NTPDase8 and CD73, co-expressed in bile canaliculi, are involved in the salvage of nucleosides from bile and the secretion of biliary electrolytes/fluid.[21]

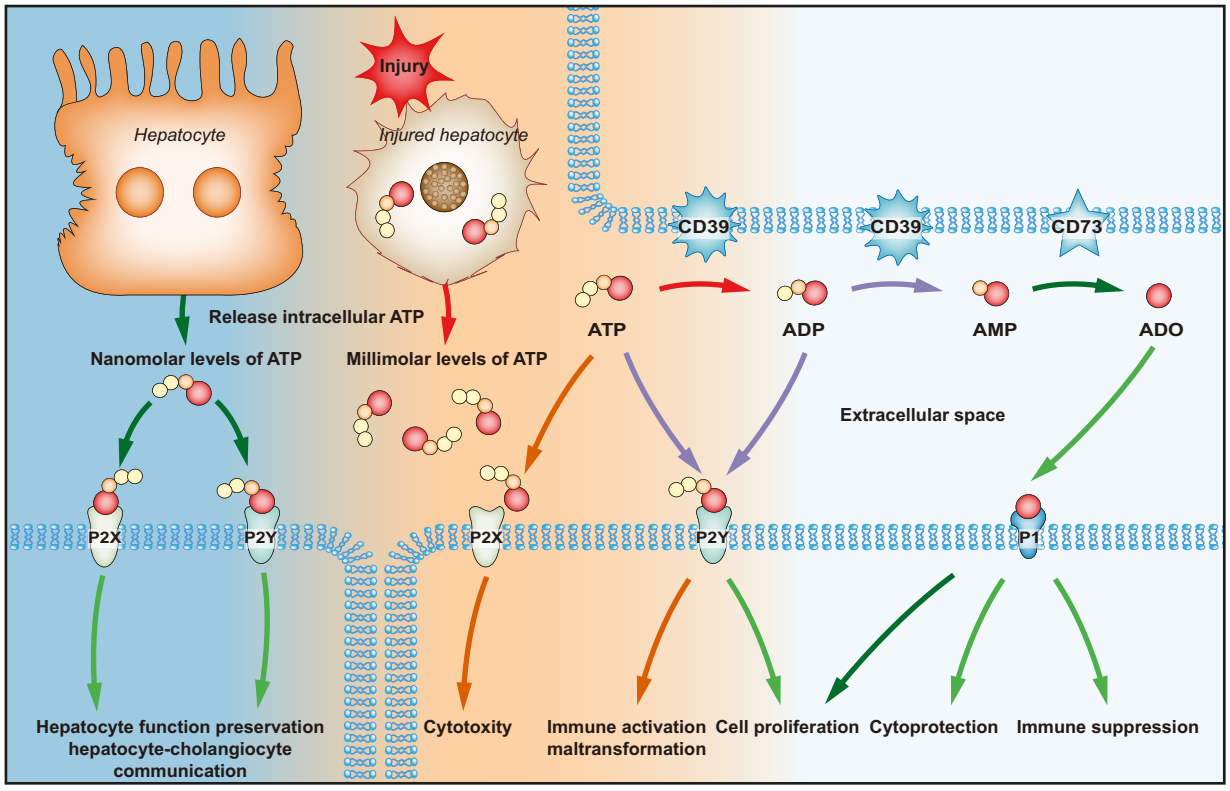

Fig. 1. Schematic summary of the main process and function of purinergic system in physiology and pathology. Under physiological conditions, nanomolar levels of ATP are essential for hepatocyte function preservation and hepatocyte-cholangiocyte communication. Upon liver injury, millimolar levels of ATP are released by the injured cells. Increased extracellular ATP exerts cytotoxic effects on the other cells via ATP-specific P2X receptors and serves as one of the danger signals to recruit immune cells to degrade ATP to ADO sequentially by CD39 and CD73. Unlike ATP, ADO, the hydrolytic product of ATP, exhibits cytoprotective and immune suppressive functions through the P1 receptor, thus terminating liver inflammation and promoting liver regeneration. ADO, adenosine; P1, purinergic type 1; P2, purinergic type 2.

Table 1. Expression and functions of purinergic receptors and ectonucleotidases in liver-resident cells.

	Nucleotide receptors	NTPDases and CD73	Adenosine receptors
Hepatocytes	$P2X_4$: Na^+ and Ca^{2+} transport; glycogen metabolism; and cell volume regulation[13] $P2X_7$: Cytotoxicity and apoptosis induction[22] $P2Y_1$: Glycogen metabolism[11] $P2Y_2$: Glycogen metabolism and cell proliferation[16] $P2Y_{13}$: Cholesterol transport,[14] lipoprotein secretion,[15] and HDL endocytosis[12]	NTPDase8: nucleoside salvage and bile secretion[21] CD73: Mallory-Denk body formation[82]	A1 and A3: Ca^{2+}-mediated ureagenesis[19] A2A: cAMP-mediated ureagenesis[19] A2B: cAMP-mediated ureagenesis,[19] glycogenolysis[20] and gluconeogenesis[20]
Cholangiocytes	$P2X_4$: Cl^- channel activation and bile secretion[18] $P2Y_2$: Na^+/H^+ exchange for bile secretion,[17] downregulation of IL-6 transcription[127] $P2Y_4$ and $P2Y_6$: downregulation of IL-6 transcription[127] $P2Y_{11}$: $cAMP/Ca^{2+}$/IL-6 mediation to promote cholangiocyte proliferation[127]	NTPDase8 and CD73: expressed on bile canaliculi[53]	A2B: Ca^{2+}-mediated IL-6 expression and protective effects for BDL motility[128]
Hepatic stellate cells and myofibroblasts	$P2X_7$: PKC/GSK3β-dependent proliferation, activation and synthesis of collagen I[49] $P2Y_6$: IP_3/Ca^{2+} signalling to promote pre-collagen transcription and cell contraction[47]	NTPDase 2: promotes cholangiocyte proliferation[129] and protects against CCl_4-induced liver injury CD73: highly expressed in myofibroblasts[53]	A2A: cAMP/PKA-regulated loss of contraction,[55] proliferation promotion and apoptosis reduction,[56] PKA/Src/MAPK/ERK-regulated collagen I production,[57] and p38 MAPK-regulated collagen III production[58]
Endothelial cells	$P2X_{1,4,5,7}$: nerve-mediated vasoconstriction[130] $P2X_7$: presentation of adhesion molecules and chemokines to recruit neutrophils[23] P2Y: contraction of hepatic portal veins[131] and vasodilatation in hepatic arteries[132] $P2Y_2$: modulation of HGF and IL-6 expression[44]	CD39 and CD73: essential for sinusoidal endothelial cells to promote liver regeneration[44] and limit inflammation[29]	A2A, A2B: enhancement of barrier protection and prevention of tissue leukocyte accumulation via cAMP[27]
Kupffer cells	$P2X_7$: release of IL-1β, PGE2, IL-6, and HMGB1 to promote inflammation.[133] $P2Y_{13}$: release of IL-6 to promote inflammation[134]	CD39 and CD73: few data available	A2: suppression of LPS-induced inflammatory cytokine secretion via cAMP/DUSP1[135]

A1, adenosine receptor A1; A2A, adenosine receptor A2A; A2B, adenosine receptor A2B; A3, adenosine receptor A3; BDL, bile duct ligation; DC(s), dendritic cells; IFN, interferon; IL-, interleukin-; LPS, lipopolysaccharide; NK, natural killer; NKT, natural killer T; NTPDases, ectonucleoside triphosphate diphosphohydrolases; P1, purinergic type 1; P2, purinergic type 2; PGE2, ROS, reactive oxygen species; TNF, tumour necrosis factor; Treg(s), regulatory T cells.

Table 2. Functions of purinergic receptors in immune cells recruited to the liver.

	Nucleotide receptor	CD39 and CD73 expression	Adenosine receptor
Neutrophils	$P2X_7$, $P2Y_2$: control of the chemotaxis effects of ATP for guiding of neutrophils to injured sites[23] $P2Y_2$: liver infiltration[97]	>90% express CD39; CD73 expressed to some extent[25]	A1, A3: promotion of chemotaxis and phagocytosis[30] A2A, A2B: inhibition of adhesion to endothelia, trafficking and effector functions[27,29,30]
Macrophages	$P2X_4$: release of HMGB1 and IL1β via ROS production[136] $P2X_7$: release of HMGB1 and IL1β via ROS production[136] and upregulation of the activity and gene expression of CD39[137]	Double-positive for CD39 and CD73[138]	A2A: termination of macrophage activation[35] and promotion of IL10 production[139] A2B: promotion of IL10 production[140] and inhibition of GCSF-dependent proliferation[36]
DCs	$P2X_7$: inflammatory cytokine secretion and antigen presentation of DCs[141] $P2Y_{11}$: DC maturation[142]	Highly express CD39 and CD73[122]	A2: limitation of DC activation and performance of immunosuppressive functions[38]
NK cells	$P2X_{3,6}$; $P2Y_{1,2,14}$: inhibition of NK cell secretion of IFN-γ, suppression of NK cell cytotoxicity, and promotion of liver regeneration[45]	$CD39^+CD73^-$ cells: modulate IFN-γ production and NK cell expansion[143]	A2: inhibition of cytotoxic activity and cytokine production[37]
T helper cells	$P2X_7$: promotion of the conversion to Th17 and inhibition of Treg function[144] $P2Y_1$: stimulation of T cell activation[145]	20∼30% of $CD4^+$ T cells express CD39, 10% of $CD4^+$ T cells express CD73[25]	A2A: inhibition of Th cell development[39] and proinflammatory cytokine production[40]
Cytotoxic T cells	$P2X_7$: blockade of the development of IL-10-producing $CD8^+$ cells[146]	5% of $CD8^+$ T cells express CD39, 50% of $CD8^+$ T cells express CD73[25]	A2A: interference with initial activation, cytokine production, metabolic activity, and effector differentiation[147]
Tregs	$P2X_7$: inhibition of Treg generation and reduction of Treg suppressive functions and cell stability[144]	$CD39^+$[25]; mouse Tregs highly express CD73, while 1%∼5% of human Tregs express CD73[25]	A2A: Promotion of expansion of Tregs and enforcement of immunosuppressive functions[32]; upregulation of CD39 and CD73 expression[34] A2B: promotion of Treg generation from $CD4^+$ naïve cells [31,33]
NKT cells	$P2X_7$: enhancement of cytokine production and exaggeration of liver injury[148]; induction of apoptosis[149]	$CD39^+CD73^+$: affects cytokine secretion and cell survival[149]	A2A: inhibition of NKT cell activation and suppression of NKT cell-triggered inflammatory responses[41]
B lymphocytes	Undefined	90% of human B lymphocytes are $CD39^+CD73^+$ with immunosuppressive functions[150]	A3: suppression of B-cell expansion and functional activation[150]

A1, adenosine receptor A1; A2A, adenosine receptor A2A; A2B, adenosine receptor A2B; A3, adenosine receptor A3; DC(s), dendritic cells; GCSF, granulocyte colony-stimulating factor; IFN, interferon; IL-, interleukin-; NK, natural killer; NKT, natural killer T; P1, purinergic type 1; P2, purinergic type 2; ROS, reactive oxygen species; TNF, tumour necrosis factor; Treg(s), regulatory T cells.

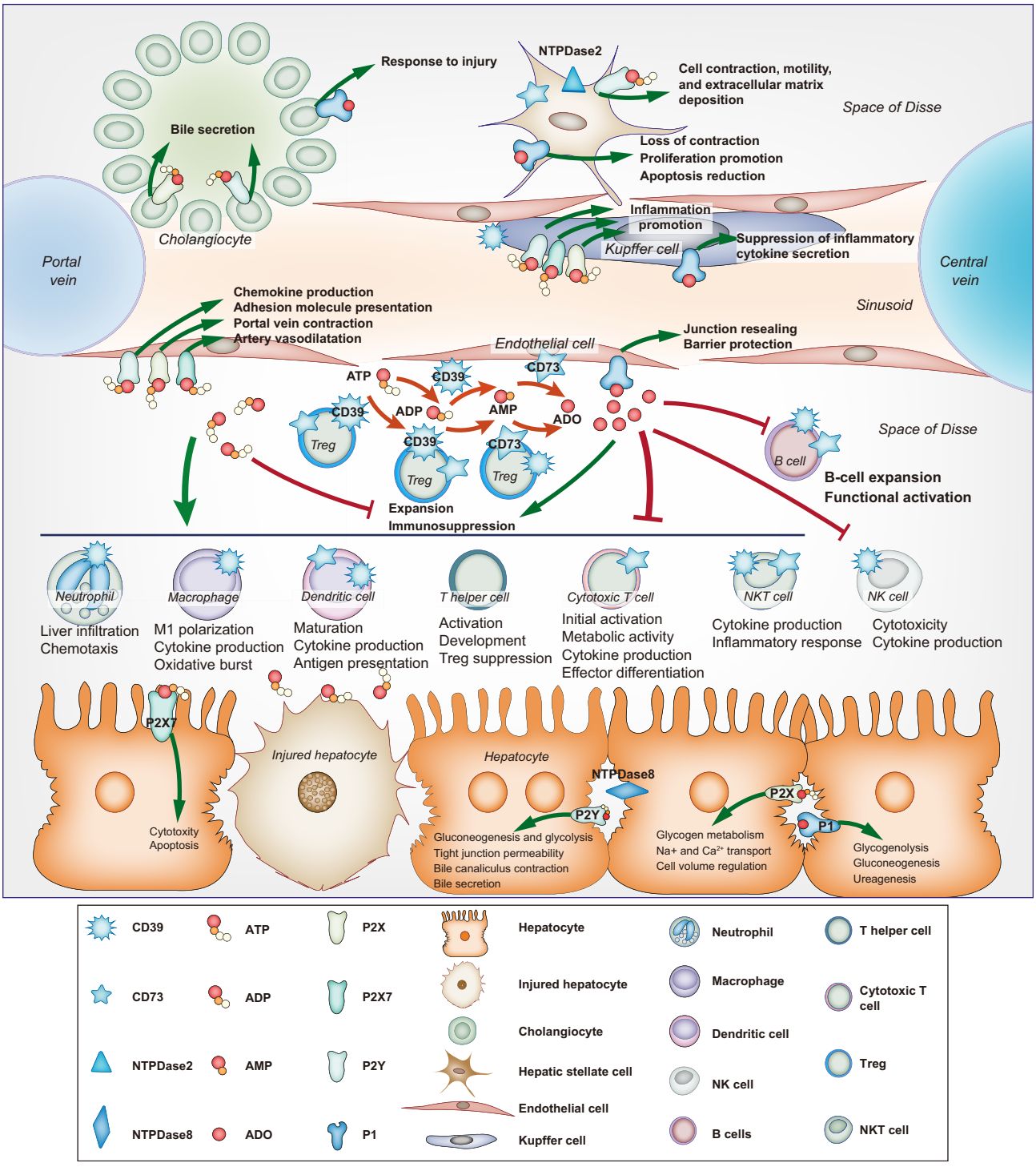

Fig. 2. Purinergic signalling in liver-resident cells and recruited immune cells upon injury. Both liver-resident cells and recruited immune cells express different purinoceptor subtypes and NTPDases, thus responding to purinergic signals and functioning as regulators of purinergic signals. Injury-increased extracellular ATP levels promote endothelial cells to produce cytokines and present adhesion molecules to recruit immune cells, stimulate Kupffer cells to promote inflammation, and activate hepatic stellate cells to myofibroblasts. Immune cells recruited to the injured liver highly express CD39 and CD73 scavenging extracellular ATP and converting it into immunosuppressive adenosine. Adenosine has negative feedback effects on the endothelial cells and immune cells, leading to reduced leucocyte recruitment and terminating inflammatory responses. ADO, adenosine; NK, natural killer; NKT, natural killer T; NTPDases, ecto-nucleoside triphosphate diphosphohydrolases; P1, purinergic type 1; P2, purinergic type 2; Tregs, regulatory T cells.

Restriction of inflammation

Upon liver injury, the increased levels of extracellular ATP exert cytotoxic effects on hepatocytes via $P2X_7$-mediated Ca^{2+}-dependent mechanisms.[22] Extracellular ATP also binds to $P2X_7$ receptors on the endothelium to produce chemokines and present adhesion molecules, including integrin αMβ2 and

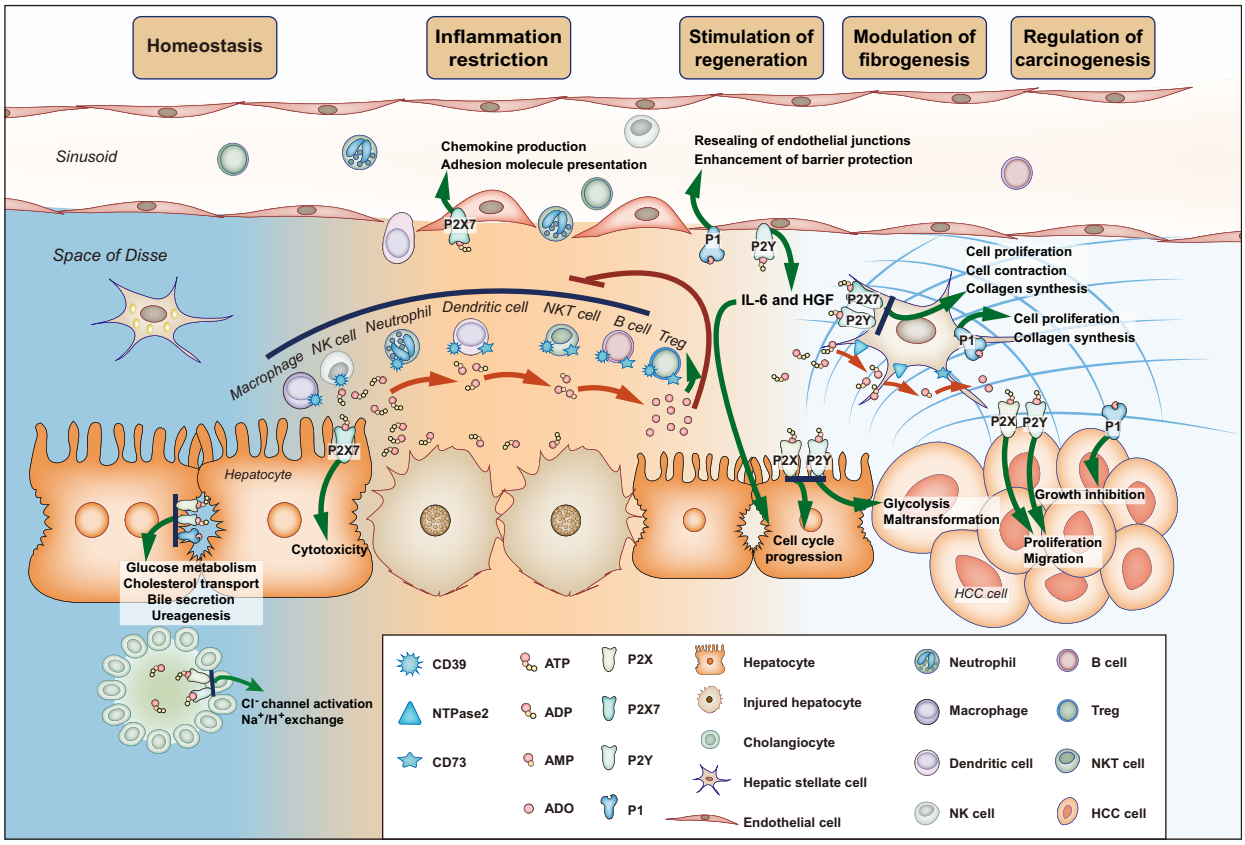

Fig. 3. Physiological and pathological functions of purinergic signals in the liver. In the normal human liver, purinergic signals are involved in liver function homeostasis. Upon liver injury, ATP is released from injured cells, and purinergic signalling orchestrates tissue repair and functional restoration via autocrine and paracrine communication between liver-resident cells and recruited immune cells. Purinergic signals, especially CD39- and CD73-mediated adenosine production, are mainly involved in restricting inflammation, stimulating regeneration, modulating fibrogenesis and regulating carcinogenesis, by orchestrating the response of liver-resident cells, including hepatocytes, endothelial cells, and hepatic stellate cells. ADO, adenosine; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; IL-6, interleukin-6; NK, natural killer; NKT, natural killer T; P1, purinergic type 1; P2, purinergic type 2; Tregs, regulatory T cells.

endothelial ligand intercellular adhesion molecule-1, in order to draw neutrophils out of the bloodstream.[23] The chemokines and cytokines produced by neutrophils further recruit other immune cells to the site of injury.[24]

Recruited immune cells expressing high levels of CD39 and CD73 scavenge extracellular ATP to generate immunosuppressive adenosine (Fig. 2). CD39 is expressed by approximately 90% of neutrophils, 90% of monocytes/macrophages, and natural killer (NK) cells, whereas CD73 is only expressed by small proportions of these cells[25]; these molecules hydrolyse ATP/ADP to AMP upon injury. CD39 and CD73 are co-expressed by sinusoidal endothelial cells, dendritic cells (DCs), regulatory T cells (Tregs), NK T (NKT) cells, and B lymphocytes, which are responsible for ATP/ADP hydrolysis and subsequent adenosine production.[25,26]

Adenosine, the hydrolytic product of ATP, has negative feedback effects on endothelial cells and immune cells, reducing leucocyte recruitment, enhancing Treg activity, and suppressing T lymphocyte activation (Fig. 3). Activation of the adenosine receptors on endothelial cells increases intracellular cAMP levels to reseal endothelial junctions and enhance barrier protection, thus preventing the accumulation of immune cells in tissue.[27,28] Adenosine functions as an anti-adhesive signal for neutrophil binding to microvascular endothelia[27,29] and inhibits neutrophil trafficking and effector functions, including granule release, oxidative bursts, and cytokine production.[30] With regard to Tregs, adenosine promotes the generation and expansion of Tregs, induces immunosuppressive functions, and upregulates CD39 and CD73 expression.[31–34]

Adenosine terminates macrophage activation via A2A/NF-κB-induced reduction of tumour necrosis factor (TNF)-α expression[35] and inhibits macrophage colony-stimulating factor-dependent proliferation of macrophages by inducing A2B/cAMP/$p27^{kip-1}$ expression.[36] Moreover, adenosine also inhibits the cytotoxic activity and cytokine production of NK cells[37] and exerts immunosuppressive effects on DCs, including reducing interleukin (IL)-12, TNF-α, C-X-C motif chemokine ligand (CXCL)-10, C-C motif chemokine ligand (CCL)-2, and CCL-12 secretion, as well as decreasing major histocompatibility complex (MHC) class II expression, and impairing allogenic T cell proliferation via the cAMP/protein kinase A (PKA) signalling pathway.[38] Furthermore, adenosine impairs the maturation of naïve T cells into Th cells[39] and reduces T cell receptor-stimulated proinflammatory cytokine production by $CD4^+$ Th cells.[40] In addition, adenosine activates A2A receptors to inhibit NKT cell activation and suppresses NKT cell-triggered inflammatory responses.[41]

Stimulation of liver regeneration

Regeneration is crucial for the restoration of hepatocyte volume and liver function. Purinergic signals participate in this process mainly by stimulating proliferation and protecting against injury. Partial

hepatectomy (PH) is the most extensively studied method for modelling liver regeneration. PH induces rapid release of ATP into the extracellular space in the remnant liver in response to mechanical stress, leading to hepatocyte cell cycle entry.[42] $P2X_4$ promotes liver regeneration following PH in mice by regulating biliary homeostasis, and *P2x4* knockout increases hepatocyte necrosis and liver cholestasis, resulting in delayed regeneration.[43] $P2Y_2$ receptors are involved in the modulation of sinusoidal endothelial cells expressing hepatocyte growth factor (HGF) and IL-6 via phosphorylation of vascular endothelial growth factor (VEGF) receptor 2 (Fig. 3).[44]

PH increases CD39 expression in vascular and sinusoidal endothelial cells in response to VEGF and induces HGF secretion which stimulates liver regeneration.[44] Depletion of CD39 induces endothelial cell apoptosis, impairs hepatocyte regeneration, and decreases overall survival post PH.[44] NK cells, which are essential for hepatic parenchymal and non-parenchymal cellular crosstalk, help promote liver regeneration by hydrolysing ATP and increasing cytotoxicity.[45] Administration of apyrase, an exogenous ATPase, can enhance hepatocyte proliferation and reduce liver injury by affecting $P2X_3$- and $P2Y_1$-activated NK cells.[45] PH also leads to the mobilisation and recruitment of $CD39^+$ haematopoietic stem cells from the bone marrow to the liver, promoting regeneration through CD39-dependent ATP hydrolysis and A2A receptor signalling.[46]

Modulation of fibrosis

Fibrosis and its end stage, cirrhosis, are common hepatopathologic conditions with different underlying aetiologies. Myofibroblasts (derived from HSCs) and portal fibroblasts are involved in extracellular matrix production in response to liver injury and play a central role in liver fibrosis.

Quiescent rat HSCs express functional $P2Y_{2,4}$ receptors in response to UTP and ATP but switch to expressing $P2Y_6$ after activation, in order to mediate pre-collagen transcription and cell contraction via UDP- and ATP-mediated inositol phosphate-3/Ca^{2+} signalling.[47] Pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonate (PPADS), a P2 receptor antagonist, can attenuate carbon tetrachloride (CCl_4)- and dimethylnitrosamine (DMN)-induced liver fibrosis by blocking P2Y/Ca^{2+} signalling-mediated extracellular matrix transcription and HSC proliferation.[48] With regard to HSC activation, the expression of $P2X_7$ receptors is significantly increased, promoting protein kinase C (PKC)/glycogen synthase kinase-3β (GSK3β)-dependent HSC proliferation and collagen production[49]; the increased expression of $P2X_4$ stimulates calcium entry and lysosomal exocytosis for ATP release, myofibroblast activation, and profibrogenic secretion.[50] In CCl_4-treated mouse liver fibrosis, $P2X_7$ expression is enhanced, and treatment with A438079, a specific $P2X_7$ antagonist, relieves liver injury, inflammatory responses, and collagen accumulation.[51] In contrast to those subjected to CCl_4-induced liver fibrosis, mice subjected to bile duct ligation (BDL) or fed a methionine- and choline-deficient diet (MCDD) exhibit increased expression of $P2X_4$. In addition, *P2x4* gene deficiency or treatment with the $P2X_4$ antagonist 5-BDBD protects mice from BDL- or MCDD-induced liver fibrosis but not CCl_4-induced liver fibrosis.[50] These data reveal that purinergic signals have different impacts on biliary and non-biliary fibrosis, and the underlying mechanisms remain unclear.

The expression of NTPDase2 is increased in activated HSCs and portal fibroblasts after CCl_4- or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-mediated induction of liver fibrosis. NTPDase2-deficient mice display CCl_4-induced liver fibrosis faster than wild-type mice, suggesting that ATP hydrolysis by myofibroblasts has protective effects.[52] CD73 is weakly expressed in quiescent HSCs and portal fibroblasts, but its expression is markedly induced in activated HSCs and portal fibroblasts after CCl_4 intoxication and BDL.[53] In contrast to NTPDase2-deficient mice, mice deficient in CD73 are protected against CCl_4- and thioacetamide (TAA)-induced liver fibrosis.[54] This may be due to low NTPDase2 expression in the myofibroblasts, but high CD73 expression in immune cells, endothelial cells, and myofibroblasts. Therefore, CD73 deficiency results in reduced adenosine production and low P2 receptor-mediated HSC proliferation and collagen expression.

In vitro studies have revealed that adenosine activates A2A receptors to inhibit cAMP/PKA/Rho-mediated HSC contraction,[55] promote cAMP-PKA/Rac1/p38 mitogen-activating protein kinase (MAPK)-mediated HSC proliferation,[56] stimulate PKA/Src- and MAPK/ERK-mediated collagen type I expression,[57] and increase p38 MAPK-mediated collagen type III expression.[58] However, the *in vivo* therapeutic effects of adenosine, its derivatives, and its receptor antagonists on liver fibrosis/cirrhosis remain controversial.

Adenosine A2A expression is increased in HSCs in TAA-induced murine liver fibrosis, and A2A depletion or treatment with the A2A-specific antagonist ZM241385 protects against TAA- or CCl_4-induced liver fibrosis/cirrhosis,[58] which is consistent with previous data on CD73 deficiency. Moreover, adenosine can attenuate CCl_4-induced rat liver fibrosis by reducing collagen deposition[59] and reverse CCl_4-induced rat cirrhosis by enhancing fibrolytic activity and hepatocyte proliferation.[60] Consistent with these findings, the aspartate salt of adenosine (IFC-305) was shown to reverse CCl_4-induced cirrhosis in rats by reducing the M1/M2 macrophage ratio and inflammatory cytokine production.[61] The potential therapeutic targets for liver fibrosis are summarised in Fig. 4; the discrepancies in these data might be attributable to the ubiquitous expression of P1 receptors and the different effects of adenosine on different cell types, such as myofibroblasts, hepatocytes, and immune cells. However, this requires further investigation.

Regulation of carcinogenesis

Carcinogenesis is closely associated with chronic inflammation of many aetiologies and often occurs on a background of cirrhosis. Large amounts of ATP are released into the extracellular space of the inflammatory tumour microenvironment and the fast-growing tumour centre. Hepatoma cells escape extracellular ATP-induced cytotoxicity via autophagy when the ATP concentration is less than 1 mM; however, apoptosis and cell death are induced when the concentration of ATP reaches 2.5 mM.[62]

In response to extracellular ATP, the expression of at least one of the P2X ($P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, or $P2X_6$) or P2Y receptors ($P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, or $P2Y_{14}$) is elevated in liver tumour tissue compared to uninvolved areas or normal liver tissue.[63–65] Among these receptors, $P2X_4$ is differentially expressed in HCV-induced hepatocellular carcinoma (HCC) and non-HCV HCC,[63] and $P2X_3$ is associated with poor recurrence-free survival in patients with HCV-induced HCC.[64] Treatment with the $P2X_3$ antagonist AF-353 (PubChem CID: 15953802) or A317491 reduces ATP-induced cell proliferation and impairs cell cycle progression. Among the P2Y receptors, $P2Y_{11}$ is highly expressed in

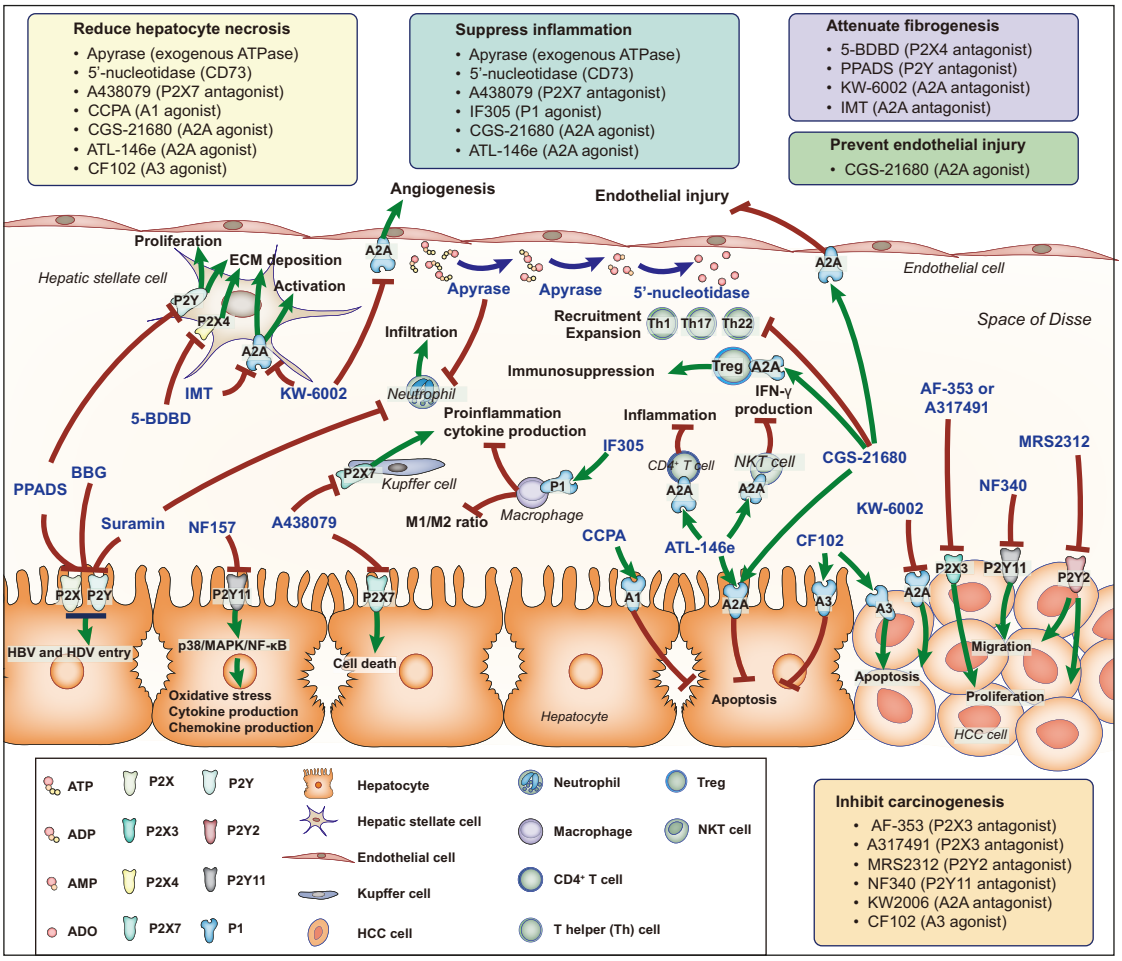

Fig. 4. Potential therapeutic targets of purine signals for liver diseases. Animal experiments reveal that ectonucleotidases (apyrase and 5′-nucleotidase), $P2X_7$ receptor antagonists (A438079), and P1 receptor agonists (CCPA, CGS-21680, ATL-146e, and CF102) can reduce hepatocyte necrosis. Similarly, ectonucleotidases (apyrase and 5′-nucleotidase), $P2X_7$ receptor antagonists (A438079), and P1 receptor agonists (IF305, CGS-21680, and ATL-146e) can attenuate inflammation. A2A agonist, CGS-21680, could prevent endothelial cell injury. However, antagonists of $P2X_4$ receptor (5-BDBD), P2Y receptor (PPADS), and A2A receptor (KW-6002 and IMT) perform the function of relieving fibrogenesis by targeting hepatic stellate cells. For HCC, P2 receptor antagonists (AF-353, A317491, MRS2312, and NF340), A2A receptor agonists (KW2006), and A3 agonist (CF102) can reduce HCC cell viability and induce HCC cell apoptosis with promising therapeutic effects. A2A, adenosine receptor A2A; A3, adenosine receptor A3; ADO, adenosine; HCC, hepatocellular carcinoma; NK, natural killer; NKT, natural killer T; P1, purinergic type 1; P2, purinergic type 2; Tregs, regulatory T cells.

HCC tissues but rarely detected in non-cancerous liver tissues.[65] Moreover, $P2Y_{11}$ contributes to ATP-induced Ca^{2+} signalling and HCC cell migration, and treatment with its antagonist (NF340) can attenuate the effects of ATP on HCC.[65] Emerging evidence shows that $P2Y_2$ is another type of P2Y receptor involved in ATP-induced HCC cell proliferation and migration.[66] In addition, selective pharmacological inhibition of $P2Y_2$ using MRS2312 can reduce the viability of HepG2, SK-Hep1, SNU449, Huh7, and Hep3B cells in a dose-dependent manner.[67]

In hepatocytes, CD39-induced purinergic signals regulate cell metabolism and proliferation, which in turn regulate carcinogenesis. In line with this notion, CD39 deficiency increases extracellular ATP concentration, thereby activating the MAPK and mTOR pathways to stimulate aerobic glycolysis and hepatocyte proliferation, leading to maltransformation.[68] Overexpression of CD39 in Tregs and increased numbers of $CD39^+Foxp3^+$ Tregs have prognostic value in HCC.[69]

CD73 is highly expressed in approximately 50% of all HCC tissues compared with paired adjacent normal tissues, and is a negative prognostic indicator for recurrence and overall survival.[70] CD73 not only promotes the proliferation, migration, and invasion of HCC cells *in vitro* but also enhances HCC growth and metastasis *in vivo*.[70,71] Mechanistically, the enzymatic activity of CD73 is required to mediate its effects in HCC, and treatment with α,β-methylene ADP (APCP), an inhibitor of CD73 activity, can partially suppress tumour growth.[70]

In an HCC cell line with high CD73 expression, A2A receptors are involved in the activation of PI3K and Akt phosphorylation, which induces HCC cell proliferation and invasion.[70] Similar to CD73 inhibition, blocking the A2A receptor with istradefylline (KW6002) inhibits tumour growth. Interestingly, co-targeting CD73 and A2A receptors with APCP and KW6002 exerts synergistic suppressive effects on HCC cells.[70] A3 receptors are also reported to be highly expressed in the tumour tissues and peripheral blood mononuclear cells of patients with HCC. In addition, treatment with CF102, an A3 receptor agonist, inhibits PI3K-NF-κB-mediated HCC cell growth and induces apoptosis in a dose-dependent manner.[72,73] A phase I/II open-label dose escalation study revealed that CF102 therapy was safe and well

Table 3. Clinical studies of drugs targeting purinergic signals or receptors.

Disease	Drugs	Target	NCT number	Phase	Status	Results
Chronic Hepatitis C	CF-102	A3 receptor agonist	NCT00790673	Phase I-II	Complete	Non-serious adverse events include palpitations, fatigue, and headache
Hepatic impairment	KW6002	A2A receptor antagonist	NCT02256033	Phase I	Complete	No result posted
NASH	CF-102	A3 receptor agonist	NCT02927314	Phase II	Complete	No result posted
HCC	CF-102	A3 receptor agonist	NCT00790218	Phase I-II	Complete	Safe and well tolerated with favourable PK characteristics in Child Pugh A and B HCC patients[74]
HCC	CF-102	A3 receptor agonist	NCT02128958	Phase II	Active, not recruiting	

A2A, adenosine receptor A2A; A3, adenosine receptor A3; HCC, hepatocellular carcinoma; NASH, non-alcoholic steatohepatitis; PK, pharmacokinetic.

Table 4. Potential therapeutic targets for liver diseases based on purinergic signals.

Animal model	Therapeutic targets based on purinergic signals
HBV hepatitis	$P2X_7$ antagonists (suramin, PPADS, BBG) reduce HBV entry into hepatocytes.[75]
	A $P2Y_{11}$ antagonist (NF157) attenuates mitochondrial dysfunction, oxidative stress, and cytokine production of hepatocytes.[76]
Alcohol-induced injury	A $P2Y_2$ antagonist (suramin) attenuates inflammation, lipid accumulation and liver injury.[85] An A1 receptor antagonist (DPCPX)[86,87] and an A2B receptor antagonist (enprofylline)[86] reduce hepatic triglyceride levels. A2A receptor antagonists (KW-6002,[89] IMT[88]) inhibit HSC activation and matrix deposition.
NAFLD/NASH	The A2A receptor agonist CGS21680 prevents hepatocyte lipotoxicity,[95] inhibits Th cell recruitment and enhances Treg function.[96]
AIH	A P2Y antagonist (suramin),[97] an A2A agonist (ATL-146e)[104] and an A3 agonist (CF102)[73] limit inflammation and enhance hepatocyte survival.
Drug-induced liver injury	A $P2X_7$ antagonist (A438079) reduces APAP-induced hepatocyte necrosis[107] and proinflammatory cytokine production of Kupffer cells.[106] An exogenous ATPase (apyrase) attenuates APAP-induced hepatocyte necrosis and neutrophil infiltration.[105,106] An A2A receptor agonist (ATL-146e) inhibits TNF-α production in GalN/LPS-induced acute liver injury.[108]
Cholestatic injury	A P2X4 antagonist (5-BDBD) attenuates BDL-liver fibrosis[50]; an A1 receptor antagonist (DPCPX) stimulates bile acid and bilirubin elimination.[112]
IR injury	Apyrase[114] and 5′-nucleotidase[115] have hepatoprotective effects. An A1 agonist (CCPA),[113] A2 receptor agonists (CGS-21680,[116,117] ATL146e[119]), and an A3 receptor agonist (CF102)[120] attenuate hepatocyte and sinusoidal endothelial cell apoptosis.
Liver transplantation	Apyrase[123] and an A2B receptor agonist (CGS21680)[125,126] have inflammatory-attenuating effects.
HCC	P2X3 antagonists (AF-353 or A317491),[64] a $P2Y_{11}$ antagonist (NF340),[65] a $P2Y_2$ antagonist (MRS2312),[67] a CD73 inhibitor (APCP),[70] an A2A receptor antagonist (KW6002),[70] and an A3 receptor agonist (CF102)[72,73] inhibit HCC growth.

A1, adenosine receptor A1; A2A, adenosine receptor A2A; A2B, adenosine receptor A2B; A3, adenosine receptor A3; AIH, autoimmune hepatitis; APAP, acetaminophen; BBG, brilliant blue G; BDL, bile duct ligation; GalN, D-galactosamine; HCC, hepatocellular carcinoma; IR, ischaemia-reperfusion; LPS, lipopolysaccharide; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; P1, purinergic type 1; P2, purinergic type 2; PGE2, prostaglandin E2; TNF, tumour necrosis factor; Treg, regulatory T cell.

tolerated, with a median overall survival of 7.8 months in patients, including those who had previously received sorafenib treatment (67%) (Table 3).[74]

There are no suitable explanations for the conflicting findings regarding the roles of adenosine in carcinogenesis (Fig. 4), and further studies are needed to reveal the therapeutic effects of adenosine receptors on hepatoma cells.

Purinergic signals in different liver diseases and potential therapeutic targets

Based on existing knowledge regarding the role of purinergic signalling in liver physiology and pathology, many targets have therapeutic implications in liver diseases of different aetiologies (Table 4). In general, ectonucleotidases, P2 receptor antagonists, and P1 receptor agonists can limit hepatocyte and endothelial cell injury, suppress inflammation, attenuate fibrosis, and inhibit carcinogenesis (Fig. 4).

Viral hepatitis

Hepatotropic viruses, which can infect and replicate in hepatocytes and destroy infected cells, are major causes of chronic liver diseases, leading to fibrosis, cirrhosis, and HCC.

Purinergic receptors are necessary for the entry of HBV and HDV into primary human hepatocytes, as evidenced by previous findings, which showed that blocking one or more of these receptors with suramin, PPADS, or brilliant blue G markedly reduces infection of cells.[75] When human MIHA hepatocytes are transfected with HBx-encoding plasmids, the expression of the $P2Y_{11}$ receptor is increased, resulting in mitochondrial dysfunction, oxidative stress, cytokine and chemokine production, and activation of the p38/MAPK and NF-κB pathways.[76] However, treatment with NF157, a specific antagonist of $P2Y_{11}$, can attenuate the effects of HBx in hepatocytes.[76] With regard to immune cells, the percentage of $CD39^+$ Tregs in peripheral blood is higher in asymptomatic HBV carriers, but lower in patients with chronic active hepatitis B or HBV-associated acute-on-chronic liver failure than in healthy controls. Furthermore, the proportions of circulating $CD39^+$ Tregs are positively correlated with serum HBV copy numbers but negatively correlated with serum alanine aminotransferase (ALT) levels.[77] In addition, $CD39^+$ Tregs accumulate in the portal areas of liver tissues in patients with chronic HBV,[77] but their functions remain unclear.

HCV RNA replication consumes ATP and reduces cytoplasmic ATP levels, but the extracellular ATP concentration is approximately 5 mM at the replication sites and 1 mM at the peripheral sites without HCV replication.[78] The NS3 helicase of HCV binds ATP, and targeting this ATP-binding site may serve as a potential therapeutic strategy for HCV.[79] Stable expression of the HCV structural protein E1E2 in Huh7 cells results in markedly increased $P2X_4$ expression,[63] and $P2X_4$ expression is significantly

higher in HCV-induced HCC than in non-HCV HCC.[80] With regard to peripheral blood mononuclear cells, $P2X_7$ expression is increased in treatment-naïve patients with chronic HCV, as well as in patients achieving a sustained virologic response, but it remains unaltered in treatment non-responders.[81] *CD73* mRNA levels in the livers of patients with HCV-related fibrosis are less than 20% of those in normal controls.[82] However, therapeutic strategies based on these findings have not yet been developed.

Therapeutic strategies for HBV and HCV have been successfully developed. A phase I/II clinical trial testing the A3 receptor agonist CF102 for HCV revealed no serious adverse effects, including palpitations, fatigue, and headache (Table 4). Based on the disease-specific expression of $P2Y_{11}$ for HBV and $P2Y_4$ for HCV, antagonists for these receptors may be used to treat patients who have progressive fibrosis despite suppression of viral replication.

Alcohol-related liver disease

Alcohol is metabolised into large amounts of toxic intermediate substances by hepatocytes. As a result, endogenous sterile danger signals (including ATP) are released from damaged hepatocytes, leading to the recruitment of macrophages and neutrophils to the liver and exacerbating liver injury and inflammation.

Alcohol significantly increases serum and liver ATP levels by directly damaging hepatocytes and inducing IL-1β production. Mice deficient in $P2X_7$ are protected from alcohol-induced liver damage.[83] Treatment with gentiopicroside, the main active secoiridoid glycoside of *Gentiana manshurica* Kitag, reduces $P2X_7$ receptor-mediated IL-1β maturation and release in ATP/lipopolysaccharide (LPS)-stimulated macrophages, and suppresses $P2X_7$-NOD-like receptor protein 3 (NLRP3) activation in mouse models of acute and chronic alcohol-induced steatohepatitis.[84] Alcohol also increases $P2Y_2$ expression in an alcohol-induced steatohepatitis mouse model, and blockade of $P2Y_2$ receptors with suramin attenuates inflammation, lipid accumulation, and liver injury, while concomitantly downregulating CD39 expression.[85]

CD39 exerts protective effects against alcohol-induced steatohepatitis by hydrolysing extracellular ATP and indirectly regulating $P2Y_2$ expression.[85] Mice deficient in CD73 are protected from ethanol-induced fatty liver after being fed an ethanol-containing liquid Lieber-DeCarli diet due to the reduction in serum ALT and hepatic triglyceride levels.[54] This may be due to reduced adenosine-mediated extracellular matrix deposition by HSCs. In accordance with this finding, treatment with the A1 receptor antagonist DPCPX and the A2B receptor antagonist enprofylline can reduce hepatic triglyceride levels.[86,87] Moreover, treatment with the A2A receptor antagonist KW6002 and a triazine derivative (IMT) can suppress ethanol-exacerbated extracellular matrix deposition by reducing HSC activation and sinusoidal angiogenesis.[88,89]

Although there are no ongoing clinical trials on purinergic signalling-based strategies to treat alcohol-related liver disease, antagonists of $P2X_7$, $P2Y_2$, A1, and A2A receptors are promising options for future clinical investigation.

Non-alcoholic fatty liver disease/non-alcoholic steatohepatitis

Globally, non-alcoholic fatty liver disease (NAFLD) and its progressive form, non-alcoholic steatohepatitis (NASH), are having an increasing impact on public health.

In NASH models of MCDD or upon co-administration of a high-fat diet (HFD) and a low dose of the environmental toxin bromodichloromethane, $P2X_7$ expression is increased in hepatocytes, Kupffer cells, and liver sinusoidal endothelial cells, which promotes oxidative stress-induced autophagy, inflammation activation, and disease progression.[90] ATP activates the $P2X_7$ receptors on Kupffer cells to enhance antigen presentation as well as TNF-α and monocyte chemotactic protein-2 production in CCl_4-treated HFD-fed obese mice.[91] $P2X_7$ receptors also participate in HSC proliferation via the glucose transporter-4 (GLUT4)/protein kinase B (Akt)/hexokinase 2 (HK2) pathway in HFD-induced NAFLD.[92] Moreover, $P2X_7$ deficiency protects against leucocyte infiltration and hepatocyte apoptosis, decreasing inflammation and fibrosis in HFD-fed mice treated with CCl_4.[90,91] This suggests that $P2X_7$ antagonists are potential therapeutic agents for the treatment of NAFLD/NASH.

DDC treatment reduces CD73 activity by 60% in detergent-soluble liver fractions. *Cd73*$^{-/-}$ mice have fewer Mallory-Denk bodies and display less cellular ballooning and steatosis after DDC feeding than wild-type mice,[82] suggesting that CD73 antagonists may have protective effects against the development of fatty liver.

A2A expression in liver cells, including hepatocytes, is higher in HFD-fed mice than in low-fat diet-fed mice.[93] Compared to normal diet feeding, HFD feeding induces severe hepatic steatohepatitis and inflammation when A2A receptors are totally disrupted or A2A expression is specifically depleted in myeloid cells.[93,94] Consistently, stimulation of A2A receptors by CGS21680 prevents hepatocyte lipotoxicity, increases the immunosuppressive activity of Tregs, and inhibits cytokine and chemokine production for proinflammatory cell recruitment and expansion, thereby ameliorating MCDD-induced NASH in rodents.[95,96]

Besides weight loss, there is no effective treatment available for patients with NASH. A phase II clinical trial testing the A3 receptor agonist CF102 for NASH was completed recently, but the results have not been published (Table 3).

Antagonists of $P2X_7$ or CD73 and agonists of A2A receptors may serve as new therapeutic options for NASH.

Autoimmune liver diseases

Autoimmune liver diseases are progressive inflammatory liver diseases caused by the breakdown of self-tolerance; they are characterised by high serum levels of autoantibodies and interface hepatitis on liver biopsy. Concanavalin A (ConA)-induced liver injury models are most commonly used to study autoimmune hepatitis (AIH), and significant ATP release can be detected as early as 2 hours post ConA injection.[97] $P2Y_2$ is the only P2 isoform whose expression is increased after ConA injection, while the expression of $P2X_{4,7}$ and $P2Y_{4,6,14}$ is reduced.[97] Deficiency of $P2Y_2$ or administration of suramin, a P2Y antagonist, can lower neutrophil infiltration and increase NF-κB-regulated hepatocyte survival, thus attenuating ConA-mediated liver damage.[97]

CD39 expression in Tregs is involved in the pathological processes of AIH and primary biliary cholangitis (PBC). Patients with AIH have lower numbers of circulating $CD4^+CD25^+$ Tregs with lower CD39 frequencies and poorer hydrolysis of exogenous ATP than healthy controls and patients with non-autoimmune and non-viral liver disorders,[98] thus exhibiting defects in CD3/CD28-stimulated self-expansion[99] and suppression of $CD4^+CD25^-$ or $CD8^+$ T cell proliferation.[100] In PBC, although the frequency of circulating Tregs is not significantly altered, liver-infiltrating Tregs display markedly reduced CD39 expression and defects in suppressive activities.[101]

Based on CD39 expression, Th17 cells can be divided into $Th17^{CD39-}$ and $Th17^{CD39+}$ cells. $Th17^{CD39+}$ cells have higher

proportions of $CD73^+$, IL-10^+, and TGF-$\beta1^+$ cells but lower frequencies of TNF-α^+ and interferon (IFN)-γ^+ cells than $Th17^{CD39-}$ cells.[102] Low levels of CD39 and A2A expression may contribute to the perpetuation of Th17 cell effector properties and thereby the pathogenesis of juvenile autoimmune liver diseases.[102]

Blocking A2A receptors with ZM241385 aggravates ConA-induced liver injury,[103] whereas activating A2A receptors with ATL-146e markedly attenuates ConA-induced liver injury.[104] Similarly, administration of the A3 agonist CF102 prevents hepatocyte necrosis by downregulating proapoptotic protein expression and limits inflammation by reducing the phosphorylation of GSK-3β and the expression of NF-κB and TNF-α, thus protecting liver tissues from ConA-induced injury.[73]

According to the guidelines, immunosuppressive medication is recommended for AIH treatment. However, studies based on purinergic signalling reveal new therapeutic targets for AIH, including $P2Y_2$ antagonists or A2A and A3 agonists, with different underlying mechanisms.

Drug-induced liver injury

Acetaminophen (APAP) overdose is the most frequent cause of acute liver failure in the United States and in European countries. Purine signalling plays an essential role in APAP-induced liver damage since large amounts of ATP are released into the extracellular area by APAP-injured necrotic hepatocytes. ATP not only directly induces cytotoxic effects on hepatocytes via P2 receptor-dependent increases in Ca^{2+} concentration,[105] but also activates the $P2X_7$ receptors on Kupffer cells to secrete the proinflammatory cytokine IL-1β.[106] Genetic deletion of *P2x7* significantly reduces serum ALT levels, liver necrosis scores, and neutrophil infiltration in APAP-induced liver injury.[106] Accordingly, treatment with the specific $P2X_7$ antagonist A438079 markedly downregulates the APAP-induced cell death pathway of hepatocytes[107] and/or damage-associated molecular pattern (DAMP)-mediated proinflammatory cytokine production by Kupffer cells.[106]

Deficiency of CD39 aggravates APAP-induced liver haemorrhage and mortality,[106] and administration of apyrase reduces neutrophil infiltration and serum ALT, as well as proinflammatory cytokine levels, thus attenuating APAP-induced liver injury.[105,106] However, treatment with the non-specific P1 receptor antagonist theophylline further increases serum ALT levels and worsens APAP-mediated liver injury,[105] while treatment with the A2A receptor agonist ATL-146e significantly inhibits D-galactosamine (GalN)- and LPS-induced acute liver injury, partially by inhibiting TNF-α production.[108]

To date, N-acetylcysteine is the only therapy proven to be effective for drug-induced liver injury. Since $P2X_7$ antagonists or A2A agonists are effective in experimental animal models of drug-induced liver injury, clinical trials could be carried out to compare their efficacy with N-acetylcysteine.

Cholestatic liver injury

Cholestasis is induced by impaired bile flow in the liver, which results in accumulation of bile constituents in the liver and blood. A lack of $P2X_4$ receptors protects mice from BDL-induced fibrosis by reducing the reactivity of bile ductules and myofibroblasts without affecting immune cells; pharmacological inhibition of $P2X_4$ with 5-BDBD can attenuate myofibroblast activation and collagen accumulation.[50]

Although NTPDase 2 expression is increased in CCl_4-treated rat livers, its expression is reduced in the portal fibroblasts of BDL rats and absent in patients with cirrhotic PBC.[109] CD39 expression is relatively low in the livers of normal individuals and patients with primary sclerosing cholangitis, but high in the immune cells of the colon; depletion of CD39 enhances biliary injury and fibrosis by affecting gut-imprinted $CD8^+$ T cells.[110] Mice deficient in CD39 display more severe biliary fibrosis after exposure to DDC than wild-type mice, mainly due to loss of CD39 expression on myeloid cells.[111] Hence, administration of CD39-expressing myeloid cells may serve as a therapeutic strategy for cholestatic diseases.

Alpha-naphthyl isothiocyanate, a hepatotoxin that induces intrahepatic cholestasis by damaging cholangiocytes and hepatocytes, increases adenosine A1 receptor expression. Deficiency of A1 receptors or treatment with the A1 antagonist DPCPX stimulates bile acid and bilirubin elimination, thus attenuating α-naphthyl isothiocyanate-induced cholestasis.[112] This suggests that A1 antagonists may exert therapeutic effects in cholestatic diseases.

For cholestatic diseases, therapies range from ursodeoxycholic acid and farnesoid X receptor agonists to peroxisome proliferator-activated receptor agonists. Strategies based on purinergic signalling, including $P2X_4$ antagonists, CD39-expressing myeloid cells, and A1 antagonists have shown therapeutic effects in experimental animal models of cholestatic disease, though clinical trials are needed to compare their effects with those of other available therapies.

Ischaemia-reperfusion injury

Ischaemia-reperfusion (IR) injury occurs in many clinical settings, including liver resections, haemorrhagic shock, and liver transplantation, wherein ATP content is reduced in mitochondria isolated from liver tissue, resulting in reactive oxygen species (ROS) generation and Ca^{2+}-induced mitochondrial swelling.[113]

Surgical application of ischaemic preconditioning (IPC), a brief period of portal triad occlusion and reperfusion before sustained IR, induces specificity protein 1 (Sp1)-dependent CD39 expression in hepatocytes.[114] However, pharmacological inhibition of CD39 with sodium polyoxotungstate (POM-1) in wild-type mice or CD39 deficiency in mice abolishes the hepatoprotective effects of IPC.[114] Similarly, pharmacologic inhibition of CD73 with APCP or deficiency of CD73 in mice increases hepatocyte necrosis after IPC.[115] Consistent with these findings, administration of apyrase or 5′-nucleotidase to wild-type mice can protect against IR-induced liver injury.[114,115]

IPC induces adenosine-mediated tissue protection against hepatic IR. IPC or treatment with the A1 agonist, 2-chloro-N6-cyclopentyladenosine (CCPA), preserves mitochondrial ATP content, reduces ROS generation, and increases the threshold of Ca^{2+}-induced mitochondrial swelling by preserving oxidative phosphorylation efficiency and downregulating the Akt(Thr^{308})/GSK-3β(Ser^9) pathway.[113] Moreover, treatment with the A2 receptor agonist CGS-21680 attenuates hepatocyte apoptosis by reducing caspase-3 activity[116] and protects sinusoidal endothelial cells against storage/reperfusion injury by stimulating adenylate cyclase activity and cAMP formation.[117] A proteome study on hepatocytes and sinusoidal endothelial cells isolated from IR mice revealed that A2A stimulation with CGS21680 rescues the pathways of carbohydrate, protein, and lipid supply and metabolism that are downregulated by IR, and increases the levels of antioxidant enzymes, including arginase, pyruvate kinase, and 3-ketoacyl-CoA thiolase, particularly in sinusoidal endothelial cells, protecting against IR injury.[118]

NKT cells initiate the inflammatory cascade in hepatic IR injury by secreting IFN-γ and recruiting neutrophils. However, blockade of NKT cell activation with NK1.1 antibodies, CD1d antibodies, or A2A agonists, such as ATL146e inhibits IFN-γ production, attenuates hepatocyte necrosis, and reduces hepatic IR injury.[119] Moreover, treatment with the adenosine A3 receptor agonist CF102 not only attenuates hepatocyte apoptosis after IR injury, but also stimulates liver regeneration.[120]

Attenuation of IR injury improves recovery from clinical surgery, and based on previous studies of purinergic signalling, clinical trials are needed to confirm whether apyrase, 5′-nucleotidase, and A2A or A3 agonists can prevent IR injury.

Liver transplantation

Liver transplantation is the most effective therapy for acute liver failure and end-stage liver diseases. Purinergic signals are extensively involved in host immunotolerance and donor graft injury. Donor livers from transgenic mice overexpressing CD39 are protected against IR injury after extended cold preservation.[121] In contrast, donor livers from *Cd39*$^{-/-}$ mice are associated with more severe graft injury, higher proinflammatory cytokine production, and shorter survival times than those from wild-type mice after allograft transplantation.[122,123] In the context of mouse MHC-mismatched orthotopic liver transplantation, CD39-deficient donor livers display severe immune-mediated liver injury and enhanced anti-donor T cell proliferation due to reduced Treg populations and enhanced IFN-γ expression on CD8^{+}IFN-γ^{+} T cells. However, administration of apyrase partially reverses the acute rejection of *Cd39*$^{-/-}$ allograft livers and prolongs survival.[123]

Plasma ATP levels are only slightly elevated in non-tolerant patients along the continuum from before withdrawal of immunosuppression up to rejection, whereas tolerant patients have much higher levels of adenosine and higher frequencies of CD39^{+} Tregs than non-tolerant patients.[124] The expression of the A2B receptor was found to be increased after reperfusion in a rat orthotopic small-for-size liver transplantation model, and activation of the A2B receptor with CGS21680 can attenuate inflammatory responses by activating NF-κB.[125,126] Hence, administration of apyrase or A2B agonists may prevent acute rejection after liver transplantation, and further clinical investigations are needed to confirm the effects of new immunosuppressive strategies.

Conclusions

Purinergic signalling plays a crucial role in maintaining liver function under physiological conditions and serves as the central regulator of danger signals, injury minimisation, and liver function restoration upon liver injury.

Regarding liver-resident cells, specific P2 receptors have been shown to be involved in different liver diseases. For example, $P2X_7$ is involved in alcohol-related liver disease, NASH, and drug-induced liver injury; $P2Y_2$ is involved in ALD and AIH; and $P2X_3$ and $P2Y_{11}$ are involved in HCC. An in-depth understanding of disease-specific purinoreceptor expression and its functions in basic cell biology will help advance clinical pharmacotherapeutic research on different liver diseases.

Regarding immune cells, circulating Tregs have been implicated in viral hepatitis and AIH pathogenesis, rejection after liver transplantation, as well as HCC recurrence and overall survival. Hence, it is important to confirm the diagnostic and prognostic value of circulating Tregs and other immune cells for liver diseases and explore the feasibility of applying these cells to therapy.

There are still some controversial data related to the roles of purinergic signals, especially adenosine, in liver fibrosis and HCC. This may be because the cell-specific functions of purinergic signals in liver-resident cells and recruited immune cells have not been completely revealed. Although therapies based on purinergic signalling to treat animal models of liver diseases show promising results, clinical trials on these drugs are limited. Therefore, further clinical investigations on the *in vivo* effects of purinergic signals are crucial to develop novel therapies for liver diseases.

Abbreviations

A1, adenosine receptor A1; A2A, adenosine receptor A2A; A2B, adenosine receptor A2B; A3, adenosine receptor A3; AIH, autoimmune hepatitis; ALT, alanine aminotransferase; APAP, acetaminophen; APCP, α,β-methylene ADP; BDL, bile duct ligation; CCl_4, carbon tetrachloride; CD73, ecto-5′-nucleotidase; ConA, concanavalin A; DCs, dendritic cells; DMN, dimethylnitrosamine; HCC, hepatocellular carcinoma; HFD, high-fat diet; HGF, hepatocyte growth factor; HSCs, hepatic stellate cells; IFN, interferon; IL-, interleukin-; IPC, ischaemic preconditioning; IR, ischaemia-reperfusion; MAPK, mitogen-activating protein kinase; MCDD, methionine- and choline-deficient diet; MHC, major histocompatibility complex; NAFLD, non-alcoholic fatty liver disease; NK, natural killer; NKT, natural killer T; NTPDases, ectonucleoside triphosphate diphosphohydrolases; PBC, primary biliary cholangitis; P1, purinergic type 1; P2, purinergic type 2; PH, partial hepatectomy; PKA, protein kinase A; PPADS, pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonate; ROS, reactive oxygen species; TAA, thioacetamide; Tregs, regulatory T cells; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

Financial support

This work was supported by the National Natural Science Foundation of China (grant numbers 81870399 and 81770598) and the National Science and Technology Major Special Project for New Drug Development (grant number 2018ZX09201016).

Conflicts of interest

P.W., J.J., and D.Z. declare no conflicts of interest. Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

P.W. and D.Z conceived and designed the study. J.D. developed the initial outline and first draft of the manuscript. All the authors worked together on subsequent drafts.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2020.100165.

References

Author names in bold designate shared co-first authorship

[1] Eltzschig HK, Sitkovsky MV, Robson SC. Purinergic signaling during inflammation. N Engl J Med 2012;367:2322–2333.

[2] Giuliani AL, Sarti AC, Di Virgilio F. Extracellular nucleotides and nucleosides as signalling molecules. Immunol Lett 2019;205:16–24.
[3] **Allard B**, **Longhi MS**, Robson SC, Stagg J. The ectonucleotidases CD39 and CD73: novel checkpoint inhibitor targets. Immunol Rev 2017; 276:121–144.
[4] Vaughn BP, Robson SC, Burnstock G. Pathological roles of purinergic signaling in the liver. J Hepatol 2012;57:916–920.
[5] Oliveira AG, Marques PE, Amaral SS, Quintao JL, Cogliati B, Dagli ML, et al. Purinergic signalling during sterile liver injury. Liver Int 2013;33:353–361.
[6] Vaughn BP, Robson SC, Longhi MS. Purinergic signaling in liver disease. Dig Dis 2014;32:516–524.
[7] Burnstock G, Vaughn B, Robson SC. Purinergic signalling in the liver in health and disease. Purinergic Signal 2014;10:51–70.
[8] Vuerich M, Robson SC, Longhi MS. Ectonucleotidases in Intestinal and hepatic inflammation. Front Immunol 2019;10:507.
[9] Velazquez-Miranda E, Diaz-Munoz M, Vazquez-Cuevas FG. Purinergic signaling in hepatic disease. Purinergic Signal 2019;15:477–489.
[10] Chari RS, Schutz SM, Haebig JE, Shimokura GH, Cotton PB, Fitz JG, et al. Adenosine nucleotides in bile. Am J Physiol 1996;270:G246–G252.
[11] Dixon CJ, Hall JF, Webb TE, Boarder MR. Regulation of rat hepatocyte function by P2Y receptors: focus on control of glycogen phosphorylase and cyclic AMP by 2-methylthioadenosine 5'-diphosphate. J Pharmacol Exp Ther 2004;311:334–341.
[12] Malaval C, Laffargue M, Barbaras R, Rolland C, Peres C, Champagne E, et al. RhoA/ROCK I signalling downstream of the P2Y13 ADP-receptor controls HDL endocytosis in human hepatocytes. Cell Signal 2009;21:120–127.
[13] Varela D, Penna A, Simon F, Eguiguren AL, Leiva-Salcedo E, Cerda O, et al. P2X4 activation modulates volume-sensitive outwardly rectifying chloride channels in rat hepatoma cells. J Biol Chem 2010;285:7566–7574.
[14] **Fabre AC**, **Malaval C**, **Ben Addi A**, Verdier C, Pons V, Serhan N, et al. P2Y13 receptor is critical for reverse cholesterol transport. Hepatology 2010;52:1477–1483.
[15] Chatterjee C, Sparks DL. Extracellular nucleotides inhibit insulin receptor signaling, stimulate autophagy and control lipoprotein secretion. PLoS One 2012;7:e36916.
[16] Tackett BC, Sun H, Mei Y, Maynard JP, Cheruvu S, Mani A, et al. P2Y2 purinergic receptor activation is essential for efficient hepatocyte proliferation in response to partial hepatectomy. Am J Physiol Gastrointest Liver Physiol 2014;307:G1073–G1087.
[17] **Zsembery A**, **Spirli C**, Granato A, LaRusso NF, Okolicsanyi L, Crepaldi G, et al. Purinergic regulation of acid/base transport in human and rat biliary epithelial cell lines. Hepatology 1998;28:914–920.
[18] Doctor RB, Matzakos T, McWilliams R, Johnson S, Feranchak AP, Fitz JG. Purinergic regulation of cholangiocyte secretion: identification of a novel role for P2X receptors. Am J Physiol Gastrointest Liver Physiol 2005;288:G779–786.
[19] Guinzberg R, Uribe S, Diaz-Cruz A, Hernandez Cruz A, Pina E. In rat hepatocytes, different adenosine receptor subtypes use different secondary messengers to increase the rate of ureagenesis. Life Sci 2006;79:382–390.
[20] Yasuda N, Inoue T, Horizoe T, Nagata K, Minami H, Kawata T, et al. Functional characterization of the adenosine receptor contributing to glycogenolysis and gluconeogenesis in rat hepatocytes. Eur J Pharmacol 2003;459:159–166.
[21] **Fausther M**, **Lecka J**, Kukulski F, Levesque SA, Pelletier J, Zimmermann H, et al. Cloning, purification, and identification of the liver canalicular ecto-ATPase as NTPDase8. Am J Physiol Gastrointest Liver Physiol 2007;292:G785–795.
[22] Zoetewij JP, van de Water B, de Bont HJ, Nagelkerke JF. The role of a purinergic P2z receptor in calcium-dependent cell killing of isolated rat hepatocytes by extracellular adenosine triphosphate. Hepatology 1996;23:858–865.
[23] McDonald B, Pittman K, Menezes GB, Hirota SA, Slaba I, Waterhouse CC, et al. Intravascular danger signals guide neutrophils to sites of sterile inflammation. Science 2010;330:362–366.
[24] Alvarenga DM, Mattos MS, Araujo AM, Antunes MM, Menezes GB. Neutrophil biology within hepatic environment. Cell Tissue Res 2018;371:589–598.
[25] Pulte ED, Broekman MJ, Olson KE, Drosopoulos JH, Kizer JR, Islam N, et al. CD39/NTPDase-1 activity and expression in normal leukocytes. Thromb Res 2007;121:309–317.
[26] Kaku H, Cheng KF, Al-Abed Y, Rothstein TL. A novel mechanism of B cell-mediated immune suppression through CD73 expression and adenosine production. J Immunol 2014;193:5904–5913.
[27] Eltzschig HK, Ibla JC, Furuta GT, Leonard MO, Jacobson KA, Enjyoji K, et al. Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors. J Exp Med 2003;198:783–796.
[28] Eltzschig HK, Weissmuller T, Mager A, Eckle T. Nucleotide metabolism and cell-cell interactions. Methods Mol Biol 2006;341:73–87.
[29] Eltzschig HK, Thompson LF, Karhausen J, Cotta RJ, Ibla JC, Robson SC, et al. Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: coordination by extracellular nucleotide metabolism. Blood 2004;104:3986–3992.
[30] Barletta KE, Ley K, Mehrad B. Regulation of neutrophil function by adenosine. Arterioscler Thromb Vasc Biol 2012;32:856–864.
[31] Romio M, Reinbeck B, Bongardt S, Huls S, Burghoff S, Schrader J. Extracellular purine metabolism and signaling of CD73-derived adenosine in murine Treg and Teff cells. Am J Physiol Cell Physiol 2011;301:C530–539.
[32] Ohta A, Kini R, Ohta A, Subramanian M, Madasu M, Sitkovsky M. The development and immunosuppressive functions of CD4(+) CD25(+) FoxP3(+) regulatory T cells are under influence of the adenosine-A2A adenosine receptor pathway. Front Immunol 2012;3:190.
[33] **Ehrentraut H**, **Westrich JA**, Eltzschig HK, Clambey ET. Adora2b adenosine receptor engagement enhances regulatory T cell abundance during endotoxin-induced pulmonary inflammation. PLoS One 2012;7:e32416.
[34] **Bao R**, **Shui X**, Hou J, Li J, Deng X, Zhu X, et al. Adenosine and the adenosine A2A receptor agonist, CGS21680, upregulate CD39 and CD73 expression through E2F-1 and CREB in regulatory T cells isolated from septic mice. Int J Mol Med 2016;38:969–975.
[35] Murphree LJ, Sullivan GW, Marshall MA, Linden J. Lipopolysaccharide rapidly modifies adenosine receptor transcripts in murine and human macrophages: role of NF-kappaB in A(2A) adenosine receptor induction. Biochem J 2005;391:575–580.
[36] **Xaus J**, **Valledor AF**, Cardo M, Marques L, Beleta J, Palacios JM, et al. Adenosine inhibits macrophage colony-stimulating factor-dependent proliferation of macrophages through the induction of p27kip-1 expression. J Immunol 1999;163:4140–4149.
[37] Lokshin A, Raskovalova T, Huang X, Zacharia LC, Jackson EK, Gorelik E. Adenosine-mediated inhibition of the cytotoxic activity and cytokine production by activated natural killer cells. Cancer Res 2006;66:7758–7765.
[38] Silva-Vilches C, Ring S, Mahnke K. ATP and its metabolite adenosine as regulators of dendritic cell activity. Front Immunol 2018;9:2581.
[39] Csoka B, Himer L, Selmeczy Z, Vizi ES, Pacher P, Ledent C, et al. Adenosine A2A receptor activation inhibits T helper 1 and T helper 2 cell development and effector function. FASEB J 2008;22:3491–3499.
[40] Hynes TR, Yost EA, Yost SM, Hartle CM, Ott BJ, Berlot CH. Inhibition of Galphas/cAMP signaling decreases TCR-stimulated IL-2 transcription in CD4(+) T helper cells. J Mol Signal 2015;10:2.
[41] **Subramanian M**, **Kini R**, Madasu M, Ohta A, Nowak M, Exley M, et al. Extracellular adenosine controls NKT-cell-dependent hepatitis induction. Eur J Immunol 2014;44:1119–1129.
[42] **Gonzales E**, **Julien B**, Serriere-Lanneau V, Nicou A, Doignon I, Lagoudakis L, et al. ATP release after partial hepatectomy regulates liver regeneration in the rat. J Hepatol 2010;52:54–62.
[43] Besnard A, Gautherot J, Julien B, Tebbi A, Garcin I, Doignon I, et al. The P2X4 purinergic receptor impacts liver regeneration after partial hepatectomy in mice through the regulation of biliary homeostasis. Hepatology 2016;64:941–953.
[44] Beldi G, Wu Y, Sun X, Imai M, Enjyoji K, Csizmadia E, et al. Regulated catalysis of extracellular nucleotides by vascular CD39/ENTPD1 is required for liver regeneration. Gastroenterology 2008;135:1751–1760.
[45] Graubardt N, Fahrner R, Trochsler M, Keogh A, Breu K, Furer C, et al. Promotion of liver regeneration by natural killer cells in a murine model is dependent on extracellular adenosine triphosphate phosphohydrolysis. Hepatology 2013;57:1969–1979.
[46] Schmelzle M, Duhme C, Junger W, Salhanick SD, Chen Y, Wu Y, et al. CD39 modulates hematopoietic stem cell recruitment and promotes liver regeneration in mice and humans after partial hepatectomy. Ann Surg 2013;257:693–701.
[47] Dranoff JA, Ogawa M, Kruglov EA, Gaca MD, Sevigny J, Robson SC, et al. Expression of P2Y nucleotide receptors and ectonucleotidases in quiescent and activated rat hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 2004;287:G417–424.

[48] Dranoff JA, Kruglov EA, Abreu-Lanfranco O, Nguyen T, Arora G, Jain D. Prevention of liver fibrosis by the purinoceptor antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate (PPADS). In Vivo 2007;21:957–965.
[49] Wu X, Wang Y, Wang S, Xu R, Lv X. Purinergic P2X7 receptor mediates acetaldehyde-induced hepatic stellate cells activation via PKC-dependent GSK3beta pathway. Int Immunopharmacol 2017;43:164–171.
[50] Le Guilcher C, Garcin I, Dellis O, Cauchois F, Tebbi A, Doignon I, et al. The P2X4 purinergic receptor regulates hepatic myofibroblast activation during liver fibrogenesis. J Hepatol 2018;69:644–653.
[51] Huang C, Yu W, Cui H, Wang Y, Zhang L, Han F, et al. P2X7 blockade attenuates mouse liver fibrosis. Mol Med Rep 2014;9:57–62.
[52] Feldbrugge L, Jiang ZG, Csizmadia E, Mitsuhashi S, Tran S, Yee EU, et al. Distinct roles of ecto-nucleoside triphosphate diphosphohydrolase-2 (NTPDase2) in liver regeneration and fibrosis. Purinergic Signal 2018;14:37–46.
[53] Fausther M, Lecka J, Soliman E, Kauffenstein G, Pelletier J, Sheung N, et al. Coexpression of ecto-5'-nucleotidase/CD73 with specific NTPDases differentially regulates adenosine formation in the rat liver. Am J Physiol Gastrointest Liver Physiol 2012;302:G447–459.
[54] Peng Z, Fernandez P, Wilder T, Yee H, Chiriboga L, Chan ES, et al. Ecto-5'-nucleotidase (CD73) -mediated extracellular adenosine production plays a critical role in hepatic fibrosis. FASEB J 2008;22:2263–2272.
[55] Sohail MA, Hashmi AZ, Hakim W, Watanabe A, Zipprich A, Groszmann RJ, et al. Adenosine induces loss of actin stress fibers and inhibits contraction in hepatic stellate cells via Rho inhibition. Hepatology 2009;49:185–194.
[56] Ahsan MK, Mehal WZ. Activation of adenosine receptor A2A increases HSC proliferation and inhibits death and senescence by down-regulation of p53 and Rb. Front Pharmacol 2014;5:69.
[57] Che J, Chan ES, Cronstein BN. Adenosine A2A receptor occupancy stimulates collagen expression by hepatic stellate cells via pathways involving protein kinase A, Src, and extracellular signal-regulated kinases 1/2 signaling cascade or p38 mitogen-activated protein kinase signaling pathway. Mol Pharmacol 2007;72:1626–1636.
[58] Chan ES, Montesinos MC, Fernandez P, Desai A, Delano DL, Yee H, et al. Adenosine A(2A) receptors play a role in the pathogenesis of hepatic cirrhosis. Br J Pharmacol 2006;148:1144–1155.
[59] Hernandez-Munoz R, Diaz-Munoz M, Suarez J, Chagoya de Sanchez V. Adenosine partially prevents cirrhosis induced by carbon tetrachloride in rats. Hepatology 1990;12:242–248.
[60] Hernandez-Munoz R, Diaz-Munoz M, Suarez-Cuenca JA, Trejo-Solis C, Lopez V, Sanchez-Sevilla L, et al. Adenosine reverses a preestablished CCl4-induced micronodular cirrhosis through enhancing collagenolytic activity and stimulating hepatocyte cell proliferation in rats. Hepatology 2001;34:677–687.
[61] Perez-Cabeza de Vaca R, Dominguez-Lopez M, Guerrero-Celis N, Rodriguez-Aguilera JR, Chagoya de Sanchez V. Inflammation is regulated by the adenosine derivative molecule, IFC-305, during reversion of cirrhosis in a CCl4 rat model. Int Immunopharmacol 2018;54:12–23.
[62] Wei Q, Zhang Y, Sun L, Jia X, Huai W, Yu C, et al. High dose of extracellular ATP switched autophagy to apoptosis in anchorage-dependent and anchorage-independent hepatoma cells. Purinergic Signal 2013;9:585–598.
[63] Manzoor S, Idrees M, Ashraf J, Mehmood A, Butt S, Fatima K, et al. Identification of ionotrophic purinergic receptors in Huh-7 cells and their response towards structural proteins of HCV genotype 3a. Virol J 2011;8:431.
[64] Maynard JP, Lee JS, Sohn BH, Yu X, Lopez-Terrada D, Finegold MJ, et al. P2X3 purinergic receptor overexpression is associated with poor recurrence-free survival in hepatocellular carcinoma patients. Oncotarget 2015;6:41162–41179.
[65] **Khalid M**, **Brisson L**, Tariq M, Hao Y, Guibon R, Fromont G, et al. Carcinoma-specific expression of P2Y11 receptor and its contribution in ATP-induced purinergic signalling and cell migration in human hepatocellular carcinoma cells. Oncotarget 2017;8:37278–37290.
[66] **Xie R**, **Xu J**, Wen G, Jin H, Liu X, Yang Y, et al. The P2Y2 nucleotide receptor mediates the proliferation and migration of human hepatocellular carcinoma cells induced by ATP. J Biol Chem 2014;289:19137–19149.
[67] Tak E, Jun DY, Kim SH, Park GC, Lee J, Hwang S, et al. Upregulation of P2Y2 nucleotide receptor in human hepatocellular carcinoma cells. J Int Med Res 2016;44:1234–1247.
[68] **Sun X**, **Han L**, Seth P, Bian S, Li L, Csizmadia E, et al. Disordered purinergic signaling and abnormal cellular metabolism are associated with development of liver cancer in Cd39/ENTPD1 null mice. Hepatology 2013;57:205–216.
[69] **Cai XY**, **Ni XC**, Yi Y, He HW, Wang JX, Fu YP, et al. Overexpression of CD39 in hepatocellular carcinoma is an independent indicator of poor outcome after radical resection. Medicine (Baltimore) 2016;95:e4989.
[70] **Ma XL**, **Shen MN**, **Hu B**, Wang BL, Yang WJ, Lv LH, et al. CD73 promotes hepatocellular carcinoma progression and metastasis via activating PI3K/AKT signaling by inducing Rap1-mediated membrane localization of P110beta and predicts poor prognosis. J Hematol Oncol 2019;12:37.
[71] **Shali S**, **Yu J**, Zhang X, Wang X, Jin Y, Su M, et al. Ecto-5'-nucleotidase (CD73) is a potential target of hepatocellular carcinoma. J Cell Physiol 2019;234:10248–10259.
[72] **Bar-Yehuda S**, **Stemmer SM**, Madi L, Castel D, Ochaion A, Cohen S, et al. The A3 adenosine receptor agonist CF102 induces apoptosis of hepatocellular carcinoma via de-regulation of the Wnt and NF-kappaB signal transduction pathways. Int J Oncol 2008;33:287–295.
[73] Cohen S, Stemmer SM, Zozulya G, Ochaion A, Patoka R, Barer F, et al. CF102 an A3 adenosine receptor agonist mediates anti-tumor and anti-inflammatory effects in the liver. J Cell Physiol 2011;226:2438–2447.
[74] Stemmer SM, Benjaminov O, Medalia G, Ciuraru NB, Silverman MH, Bar-Yehuda S, et al. CF102 for the treatment of hepatocellular carcinoma: a phase I/II, open-label, dose-escalation study. Oncologist 2013;18:25–26.
[75] Taylor JM, Han Z. Purinergic receptor functionality is necessary for infection of human hepatocytes by hepatitis delta virus and hepatitis B virus. PLoS One 2010;5:e15784.
[76] Lei C, Fan Y, Peng X, Gong X, Shao L. P2Y11R regulates cytotoxicity of HBV X protein (HBx) in human normal hepatocytes. Am J Transl Res 2019;11:2765–2774.
[77] **Tang Y**, **Jiang L**, Zheng Y, Ni B, Wu Y. Expression of CD39 on FoxP3+ T regulatory cells correlates with progression of HBV infection. BMC Immunol 2012;13:17.
[78] Ando T, Imamura H, Suzuki R, Aizaki H, Watanabe T, Wakita T, et al. Visualization and measurement of ATP levels in living cells replicating hepatitis C virus genome RNA. PLoS Pathog 2012;8:e1002561.
[79] Palla M, Chen CP, Zhang Y, Li J, Ju J, Liao JC. Mechanism of flexibility control for ATP access of hepatitis C virus NS3 helicase. J Biomol Struct Dyn 2013;31:129–141.
[80] Khalid M, Manzoor S, Ahmad H, Asif A, Bangash TA, Latif A, et al. Purinoceptor expression in hepatocellular virus (HCV)-induced and non-HCV hepatocellular carcinoma: an insight into the proviral role of the P2X4 receptor. Mol Biol Rep 2018;45:2625–2630.
[81] Ashraf W, Manzoor S, Ashraf J, Ahmed QL, Khalid M, Tariq M, et al. Transcript analysis of P2X receptors in PBMCs of chronic HCV patients: an insight into antiviral treatment response and HCV-induced pathogenesis. Viral Immunol 2013;26:343–350.
[82] Snider NT, Griggs NW, Singla A, Moons DS, Weerasinghe SV, Lok AS, et al. CD73 (ecto-5'-nucleotidase) hepatocyte levels differ across mouse strains and contribute to mallory-denk body formation. Hepatology 2013;58:1790–1800.
[83] Iracheta-Vellve A, Petrasek J, Satishchandran A, Gyongyosi B, Saha B, Kodys K, et al. Inhibition of sterile danger signals, uric acid and ATP, prevents inflammasome activation and protects from alcoholic steatohepatitis in mice. J Hepatol 2015;63:1147–1155.
[84] **Li X**, **Zhang Y**, Jin Q, Xia KL, Jiang M, Cui BW, et al. Liver kinase B1/AMP-activated protein kinase-mediated regulation by gentiopicroside ameliorates P2X7 receptor-dependent alcoholic hepatosteatosis. Br J Pharmacol 2018;175:1451–1470.
[85] Liu ZN, Jia WQ, Jiang T, Dai JW, Shuai C, Lv XW. Regulation of CD39 expression in ATP-P2Y2R-mediated alcoholic liver steatosis and inflammation. Int Immunopharmacol 2019;77:105915.
[86] Peng Z, Borea PA, Varani K, Wilder T, Yee H, Chiriboga L, et al. Adenosine signaling contributes to ethanol-induced fatty liver in mice. J Clin Invest 2009;119:582–594.
[87] **Yang P**, **Wang Z**, Zhan Y, Wang T, Zhou M, Xia L, et al. Endogenous A1 adenosine receptor protects mice from acute ethanol-induced hepatotoxicity. Toxicology 2013;309:100–106.
[88] Szuster-Ciesielska A, Sztanke K, Kandefer-Szerszen M. A novel fused 1,2,4-triazine aryl derivative as antioxidant and nonselective antagonist of adenosine A(2A) receptors in ethanol-activated liver stellate cells. Chem Biol Interact 2012;195:18–24.
[89] Chiang DJ, Roychowdhury S, Bush K, McMullen MR, Pisano S, Niese K, et al. Adenosine 2A receptor antagonist prevented and reversed liver fibrosis in a mouse model of ethanol-exacerbated liver fibrosis. PLoS One 2013;8:e69114.
[90] Das S, Seth RK, Kumar A, Kadiiska MB, Michelotti G, Diehl AM, et al. Purinergic receptor X7 is a key modulator of metabolic oxidative stress-mediated autophagy and inflammation in experimental nonalcoholic

steatohepatitis. Am J Physiol Gastrointest Liver Physiol 2013;305:G950–G963.
[91] Chatterjee S, Rana R, Corbett J, Kadiiska MB, Goldstein J, Mason RP. P2X7 receptor-NADPH oxidase axis mediates protein radical formation and Kupffer cell activation in carbon tetrachloride-mediated steatohepatitis in obese mice. Free Radic Biol Med 2012;52:1666–1679.
[92] **Chandrashekaran V**, **Das S**, Seth RK, Dattaroy D, Alhasson F, Michelotti G, et al. Purinergic receptor X7 mediates leptin induced GLUT4 function in stellate cells in nonalcoholic steatohepatitis. Biochim Biophys Acta 2016;1862:32–45.
[93] **Cai Y**, **Li H**, **Liu M**, Pei Y, Zheng J, Zhou J, et al. Disruption of adenosine 2A receptor exacerbates NAFLD through increasing inflammatory responses and SREBP1c activity. Hepatology 2018;68:48–61.
[94] **Zhou J**, **Li H**, Cai Y, Ma L, Mathews D, Lu B, et al. Mice lacking adenosine 2A receptor reveal increased severity of MCD-induced NASH. J Endocrinol 2019;243:199–209.
[95] Imarisio C, Alchera E, Sutti S, Valente G, Boccafoschi F, Albano E, et al. Adenosine A(2a) receptor stimulation prevents hepatocyte lipotoxicity and non-alcoholic steatohepatitis (NASH) in rats. Clin Sci (Lond) 2012;123:323–332.
[96] **Alchera E**, **Rolla S**, **Imarisio C**, Bardina V, Valente G, Novelli F, et al. Adenosine A2a receptor stimulation blocks development of nonalcoholic steatohepatitis in mice by multilevel inhibition of signals that cause immunolipotoxicity. Transl Res 2017;182:75–87.
[97] Ayata CK, Ganal SC, Hockenjos B, Willim K, Vieira RP, Grimm M, et al. Purinergic P2Y(2) receptors promote neutrophil infiltration and hepatocyte death in mice with acute liver injury. Gastroenterology 2012;143:1620–1629.e4.
[98] Grant CR, Liberal R, Holder BS, Cardone J, Ma Y, Robson SC, et al. Dysfunctional CD39(POS) regulatory T cells and aberrant control of T-helper type 17 cells in autoimmune hepatitis. Hepatology 2014;59:1007–1015.
[99] **Longhi MS**, **Ma Y**, Bogdanos DP, Cheeseman P, Mieli-Vergani G, Vergani D. Impairment of CD4(+)CD25(+) regulatory T-cells in autoimmune liver disease. J Hepatol 2004;41:31–37.
[100] Longhi MS, Hussain MJ, Mitry RR, Arora SK, Mieli-Vergani G, Vergani D, et al. Functional study of CD4+CD25+ regulatory T cells in health and autoimmune hepatitis. J Immunol 2006;176:4484–4491.
[101] **Bernuzzi F**, **Fenoglio D**, Battaglia F, Fravega M, Gershwin ME, Indiveri F, et al. Phenotypical and functional alterations of CD8 regulatory T cells in primary biliary cirrhosis. J Autoimmun 2010;35:176–180.
[102] Liberal R, Grant CR, Ma Y, Csizmadia E, Jiang ZG, Heneghan MA, et al. CD39 mediated regulation of Th17-cell effector function is impaired in juvenile autoimmune liver disease. J Autoimmun 2016;72:102–112.
[103] Chouker A, Thiel M, Lukashev D, Ward JM, Kaufmann I, Apasov S, et al. Critical role of hypoxia and A2A adenosine receptors in liver tissue-protecting physiological anti-inflammatory pathway. Mol Med 2008;14:116–123.
[104] **Odashima M**, **Otaka M**, Jin M, Horikawa Y, Matsuhashi T, Ohba R, et al. A selective adenosine A2A receptor agonist, ATL-146e, prevents concanavalin A-induced acute liver injury in mice. Biochem Biophys Res Commun 2006;347:949–954.
[105] **Amaral SS**, **Oliveira AG**, Marques PE, Quintao JL, Pires DA, Resende RR, et al. Altered responsiveness to extracellular ATP enhances acetaminophen hepatotoxicity. Cell Commun Signal 2013;11:10.
[106] **Hoque R**, **Sohail MA**, Salhanick S, Malik AF, Ghani A, Robson SC, et al. P2X7 receptor-mediated purinergic signaling promotes liver injury in acetaminophen hepatotoxicity in mice. Am J Physiol Gastrointest Liver Physiol 2012;302:G1171–G1179.
[107] **Xie Y**, **Williams CD**, McGill MR, Lebofsky M, Ramachandran A, Jaeschke H. Purinergic receptor antagonist A438079 protects against acetaminophen-induced liver injury by inhibiting p450 isoenzymes, not by inflammasome activation. Toxicol Sci 2013;131:325–335.
[108] Odashima M, Otaka M, Jin M, Komatsu K, Wada I, Matsuhashi T, et al. Selective A2A adenosine agonist ATL-146e attenuates acute lethal liver injury in mice. J Gastroenterol 2005;40:526–529.
[109] Dranoff JA, Kruglov EA, Toure J, Braun N, Zimmermann H, Jain D, et al. Ectonucleotidase NTPDase2 is selectively down-regulated in biliary cirrhosis. J Investig Med 2004;52:475–482.
[110] **Peng ZW**, **Rothweiler S**, Wei G, Ikenaga N, Liu SB, Sverdlov DY, et al. The ectonucleotidase ENTPD1/CD39 limits biliary injury and fibrosis in mouse models of sclerosing cholangitis. Hepatol Commun 2017;1:957–972.
[111] Rothweiler S, Feldbrugge L, Jiang ZG, Csizmadia E, Longhi MS, Vaid K, et al. Selective deletion of ENTPD1/CD39 in macrophages exacerbates biliary fibrosis in a mouse model of sclerosing cholangitis. Purinergic Signal 2019;15:375–385.
[112] Yang P, Chen P, Wang T, Zhan Y, Zhou M, Xia L, et al. Loss of A(1) adenosine receptor attenuates alpha-naphthylisothiocyanate-induced cholestatic liver injury in mice. Toxicol Sci 2013;131:128–138.
[113] Duarte FV, Amorim JA, Varela AT, Teodoro JS, Gomes AP, Cunha RA, et al. Adenosine receptors: regulatory players in the preservation of mitochondrial function induced by ischemic preconditioning of rat liver. Purinergic Signal 2017;13:179–190.
[114] Hart ML, Gorzolla IC, Schittenhelm J, Robson SC, Eltzschig HK. SP1-dependent induction of CD39 facilitates hepatic ischemic preconditioning. J Immunol 2010;184:4017–4024.
[115] Hart ML, Much C, Gorzolla IC, Schittenhelm J, Kloor D, Stahl GL, et al. Extracellular adenosine production by ecto-5'-nucleotidase protects during murine hepatic ischemic preconditioning. Gastroenterology 2008;135:1739–1750.e3.
[116] **Ben-Ari Z**, **Pappo O**, Sulkes J, Cheporko Y, Vidne BA, Hochhauser E. Effect of adenosine A2A receptor agonist (CGS) on ischemia/reperfusion injury in isolated rat liver. Apoptosis 2005;10:955–962.
[117] Arai M, Thurman RG, Lemasters JJ. Contribution of adenosine A(2) receptors and cyclic adenosine monophosphate to protective ischemic preconditioning of sinusoidal endothelial cells against Storage/Reperfusion injury in rat livers. Hepatology 2000;32:297–302.
[118] Mandili G, Alchera E, Merlin S, Imarisio C, Chandrashekar BR, Riganti C, et al. Mouse hepatocytes and LSEC proteome reveal novel mechanisms of ischemia/reperfusion damage and protection by A2aR stimulation. J Hepatol 2015;62:573–580.
[119] Lappas CM, Day YJ, Marshall MA, Engelhard VH, Linden J. Adenosine A2A receptor activation reduces hepatic ischemia reperfusion injury by inhibiting CD1d-dependent NKT cell activation. J Exp Med 2006;203:2639–2648.
[120] Ohana G, Cohen S, Rath-Wolfson L, Fishman P. A3 adenosine receptor agonist, CF102, protects against hepatic ischemia/reperfusion injury following partial hepatectomy. Mol Med Rep 2016;14:4335–4341.
[121] **Pommey S**, **Lu B**, McRae J, Stagg J, Hill P, Salvaris E, et al. Liver grafts from CD39-overexpressing rodents are protected from ischemia reperfusion injury due to reduced numbers of resident CD4+ T cells. Hepatology 2013;57:1597–1606.
[122] Yoshida O, Kimura S, Jackson EK, Robson SC, Geller DA, Murase N, et al. CD39 expression by hepatic myeloid dendritic cells attenuates inflammation in liver transplant ischemia-reperfusion injury in mice. Hepatology 2013;58:2163–2175.
[123] Yoshida O, Dou L, Kimura S, Yokota S, Isse K, Robson SC, et al. CD39 deficiency in murine liver allografts promotes inflammatory injury and immune-mediated rejection. Transpl Immunol 2015;32:76–83.
[124] Baroja-Mazo A, Revilla-Nuin B, de Bejar A, Martinez-Alarcon L, Herrero JI, El-Tayeb A, et al. Extracellular adenosine reversibly inhibits the activation of human regulatory T cells and negatively influences the achievement of the operational tolerance in liver transplantation. Am J Transplant 2019;19:48–61.
[125] Tang LM, Wang YP, Wang K, Pu LY, Zhang F, Li XC, et al. Protective effect of adenosine A2A receptor activation in small-for-size liver transplantation. Transpl Int 2007;20:93–101.
[126] Tang LM, Zhu JF, Wang F, Qian J, Zhu J, Mo Q, et al. Activation of adenosine A2A receptor attenuates inflammatory response in a rat model of small-for-size liver transplantation. Transplant Proc 2010;42:1915–1920.
[127] Yu J, Sheung N, Soliman EM, Spirli C, Dranoff JA. Transcriptional regulation of IL-6 in bile duct epithelia by extracellular ATP. Am J Physiol Gastrointest Liver Physiol 2009;296:G563–G571.
[128] Lavoie EG, Fausther M, Goree JR, Dranoff JA. The cholangiocyte adenosine-IL-6 axis regulates survival during biliary cirrhosis. Gene Expr 2017;17:327–340.
[129] Jhandier MN, Kruglov EA, Lavoie EG, Sevigny J, Dranoff JA. Portal fibroblasts regulate the proliferation of bile duct epithelia via expression of NTPDase2. J Biol Chem 2005;280:22986–22992.
[130] Phillips JK, McLean AJ, Hill CE. Receptors involved in nerve-mediated vasoconstriction in small arteries of the rat hepatic mesentery. Br J Pharmacol 1998;124:1403–1412.
[131] Minamiyama Y, Takemura S, Kawada N, Inoue M. Role of nitric oxide in extracellular nucleotide-induced contractile status of assorted vessels including parts of the portal vasculature. J Hepatol 1998;28:314–319.

[132] Ralevic V, Mathie RT, Alexander B, Burnstock G. Characterization of P2X- and P2Y-purinoceptors in the rabbit hepatic arterial vasculature. Br J Pharmacol 1991;103:1108–1113.
[133] Toki Y, Takenouchi T, Harada H, Tanuma S, Kitani H, Kojima S, et al. Extracellular ATP induces P2X7 receptor activation in mouse Kupffer cells, leading to release of IL-1beta, HMGB1, and PGE2, decreased MHC class I expression and necrotic cell death. Biochem Biophys Res Commun 2015;458:771–776.
[134] Ishimaru M, Yusuke N, Tsukimoto M, Harada H, Takenouchi T, Kitani H, et al. Purinergic signaling via P2Y receptors up-mediates IL-6 production by liver macrophages/Kupffer cells. J Toxicol Sci 2014;39:413–423.
[135] Reinstein LJ, Lichtman SN, Currin RT, Wang J, Thurman RG, Lemasters JJ. Suppression of lipopolysaccharide-stimulated release of tumor necrosis factor by adenosine: evidence for A2 receptors on rat Kupffer cells. Hepatology 1994;19:1445–1452.
[136] **Kawano A**, **Tsukimoto M**, Mori D, Noguchi T, Harada H, Takenouchi T, et al. Regulation of P2X7-dependent inflammatory functions by P2X4 receptor in mouse macrophages. Biochem Biophys Res Commun 2012;420:102–107.
[137] Savio LEB, de Andrade Mello P, Figliuolo VR, de Avelar Almeida TF, Santana PT, Oliveira SDS, et al. CD39 limits P2X7 receptor inflammatory signaling and attenuates sepsis-induced liver injury. J Hepatol 2017;67:716–726.
[138] Hamidzadeh K, Mosser DM. Purinergic signaling to terminate TLR responses in macrophages. Front Immunol 2016;7:74.
[139] Csoka B, Nemeth ZH, Virag L, Gergely P, Leibovich SJ, Pacher P, et al. A2A adenosine receptors and C/EBPbeta are crucially required for IL-10 production by macrophages exposed to Escherichia coli. Blood 2007;110:2685–2695.
[140] Nemeth ZH, Lutz CS, Csoka B, Deitch EA, Leibovich SJ, Gause WC, et al. Adenosine augments IL-10 production by macrophages through an A2B receptor-mediated posttranscriptional mechanism. J Immunol 2005;175:8260–8270.
[141] Mutini C, Falzoni S, Ferrari D, Chiozzi P, Morelli A, Baricordi OR, et al. Mouse dendritic cells express the P2X7 purinergic receptor: characterization and possible participation in antigen presentation. J Immunol 1999;163:1958–1965.
[142] Wilkin F, Duhant X, Bruyns C, Suarez-Huerta N, Boeynaems JM, Robaye B. The P2Y11 receptor mediates the ATP-induced maturation of human monocyte-derived dendritic cells. J Immunol 2001;166:7172–7177.
[143] Beldi G, Banz Y, Kroemer A, Sun X, Wu Y, Graubardt N, et al. Deletion of CD39 on natural killer cells attenuates hepatic ischemia/reperfusion injury in mice. Hepatology 2010;51:1702–1711.
[144] Schenk U, Frascoli M, Proietti M, Geffers R, Traggiai E, Buer J, et al. ATP inhibits the generation and function of regulatory T cells through the activation of purinergic P2X receptors. Sci Signal 2011;4:ra12.
[145] **Woehrle T**, **Ledderose C**, Rink J, Slubowski C, Junger WG. Autocrine stimulation of P2Y1 receptors is part of the purinergic signaling mechanism that regulates T cell activation. Purinergic Signal 2019;15(2):127–137.
[146] Noble A, Mehta H, Lovell A, Papaioannou E, Fairbanks L. IL-12 and IL-4 activate a CD39-dependent intrinsic peripheral tolerance mechanism in CD8(+) T cells. Eur J Immunol 2016;46:1438–1448.
[147] **Linnemann C**, **Schildberg FA**, **Schurich A**, Diehl L, Hegenbarth SI, Endl E, et al. Adenosine regulates CD8 T-cell priming by inhibition of membrane-proximal T-cell receptor signalling. Immunology 2009;128:e728–e737.
[148] Kawamura H, Aswad F, Minagawa M, Govindarajan S, Dennert G. P2X7 receptors regulate NKT cells in autoimmune hepatitis. J Immunol 2006;176:2152–2160.
[149] Beldi G, Wu Y, Banz Y, Nowak M, Miller L, Enjyoji K, et al. Natural killer T cell dysfunction in CD39-null mice protects against concanavalin A-induced hepatitis. Hepatology 2008;48:841–852.
[150] **Saze Z**, **Schuler PJ**, Hong CS, Cheng D, Jackson EK, Whiteside TL. Adenosine production by human B cells and B cell-mediated suppression of activated T cells. Blood 2013;122:9–18.