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REVIEW

Cell type- and tissue-specific functions of ecto-5′-nucleotidase (CD73)

Marquet Minor, Karel P. Alcedo, Rachel A. Battaglia, and Natasha T. Snider
Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

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Minor M, Alcedo KP, Battaglia RA, Snider NT. Cell type- and tissue-specific functions of ecto-5′-nucleotidase (CD73). *Am J Physiol Cell Physiol* 317: C1079–C1092, 2019. First published August 28, 2019; doi:10.1152/ajpcell.00285.2019.— Ecto-5′-nucleotidase [cluster of differentiation 73 (CD73)] is a ubiquitously expressed glycosylphosphatidylinositol-anchored glycoprotein that converts extracellular adenosine 5′-monophosphate to adenosine. Anti-CD73 inhibitory antibodies are currently undergoing clinical testing for cancer immunotherapy. However, many protective physiological functions of CD73 need to be taken into account for new targeted therapies. This review examines CD73 functions in multiple organ systems and cell types, with a particular focus on novel findings from the last 5 years. Missense loss-of-function mutations in the CD73-encoding gene *NT5E* cause the rare disease "arterial calcifications due to deficiency of CD73." Aside from direct human disease involvement, cellular and animal model studies have revealed key functions of CD73 in tissue homeostasis and pathology across multiple organ systems. In the context of the central nervous system, CD73 is antinociceptive and protects against inflammatory damage, while also contributing to age-dependent decline in cortical plasticity. CD73 preserves barrier function in multiple tissues, a role that is most evident in the respiratory system, where it inhibits endothelial permeability in an adenosine-dependent manner. CD73 has important cardioprotective functions during myocardial infarction and heart failure. Under ischemia-reperfusion injury conditions, rapid and sustained induction of CD73 confers protection in the liver and kidney. In some cases, the mechanism by which CD73 mediates tissue injury is less clear. For example, CD73 has a promoting role in liver fibrosis but is protective in lung fibrosis. Future studies that integrate CD73 regulation and function at the cellular level with physiological responses will improve its utility as a disease target.

extracellular adenosine; fibrosis; hypoxia; inflammation; ischemia-reperfusion

INTRODUCTION

Coined in the 1930s, the term 5′-nucleotidase (5′-NT) refers to the enzymatic dephosphorylation of nucleoside 5′-monophosphates, such as adenosine 5′-monophosphate (AMP) (44). This activity is critical for purine salvage and purinergic signaling and can occur inside the cell or in the extracellular space (50, 124). Ecto-5′-nucleotidase, encoded by the *NT5E* gene, is the major enzyme catalyzing the formation of extracellular adenosine from AMP (124). This enzyme was designated cluster of differentiation (CD) 73 in 1989 following the characterization of three different antibodies that immunoprecipitated a 69-kDa protein from the human myeloma cell line U266 and bound similarly to human lymphocytes (109). Since then, both ecto-5′-nucleotidase and CD73 have been used to describe the same gene product (herein we refer to the protein as CD73).

CD73 regulates tissue homeostasis and pathophysiological responses related to immunity, inflammation, and cancer (8, 10, 29, 87), and CD73-targeting investigational antibodies (BMS-986179, CPI-006, MEDI9447, NZV930, and TJ004309) are currently undergoing clinical testing for advanced solid tumors (47, 81). Development of small-molecule inhibitors of CD73 is also an active area of research (54). Although the immunomodulatory and cancer-associated properties of CD73 have garnered the majority of scientific interest in recent years, *NT5E*/CD73 is ubiquitously expressed (Fig. 1) and regulates critical functions across multiple organ systems through its activity on specific cell types (29). Some of the major cell type-specific functions of CD73 include support of epithelial cell transport and tissue barrier function (19, 29), inhibition of endothelial permeability (108), reinforcement of lymphocyte-endothelium interactions (2), inhibition of macrophage- and mesenchymal cell-mediated inflammation (72, 92), hyperpolarization and relaxation of smooth muscle cells (74), and antinociception via modulation of neuronal activity (102).

The primary focus of this review is to highlight known and emerging functions of CD73 in the central nervous system (CNS), cardiovascular system, and epithelial tissues (lung,

Address for reprint requests and other correspondence: N. T. Snider, Dept. of Cell Biology and Physiology, University of North Carolina-Chapel Hill, 5340C MBRB, 111 Mason Farm Rd., Chapel Hill, NC 27516 (e-mail: ntsnider@med.unc.edu).

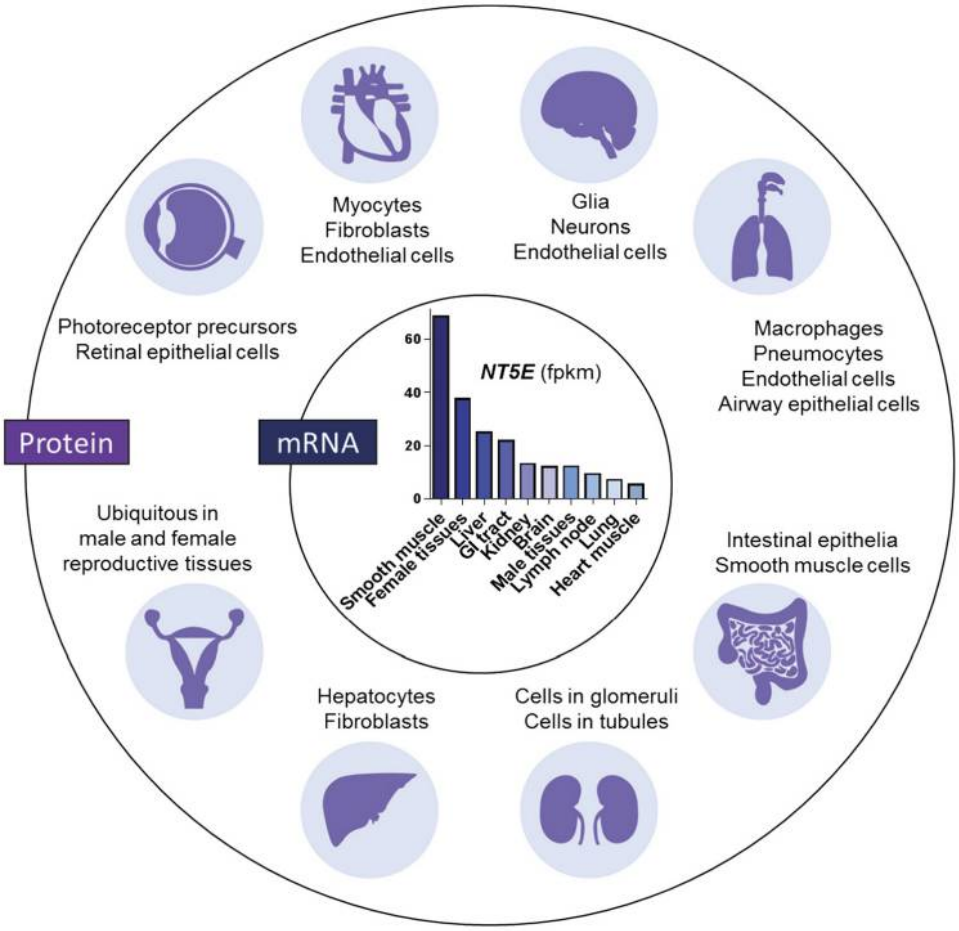

Fig. 1. Tissue-specific expression of human cluster of differentiation 73 (CD73). Outer circle depicts various tissues and cell types where CD73 protein has been detected via immunohistochemical methods, as reported by the Human Protein Atlas project. Note that expression in the eye is based on literature reports. Inner circle depicts human CD73-encoding gene *NT5E* expression (in order of abundance) based on Human Protein Atlas data. Average fragments per kilobase of transcript per million mapped reads (fpkm) values are shown for larger organ systems [e.g., gastrointestinal (GI) tract)].

liver, and kidney), with a particular emphasis on studies from the past 5 years. A comprehensive understanding of the physiological functions of CD73 is critical for further progress on the basic biology, disease mechanisms, and therapeutic targeting of this important molecule.

Molecular Functions of CD73

CD73 is a complex molecule that undergoes *N*-linked glycosylation (5), disulfide bond-mediated homodimerization, and membrane association via a glycosylphosphatidylinositol (GPI) anchor (58) (Fig. 2). Its enzymatic activity is closely coupled to that of ectoapyrase (CD39), which generates the AMP substrate for CD73 (8). Extracellular adenosine produced by CD73 acts on adenosine receptors (A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R) to activate downstream G protein-coupled signaling and modulate adenylate cyclase (AC) activity (7, 26, 39, 42), or it can be taken up into the cell via equilibrative nucleoside transporters (78). Phospholipase C (activated by A_1R, $A_{2B}R$, and A_3R), MAP kinase (activated by all 4 adenosine receptor subtypes), phosphatidylinositol 3-kinase (activated by A_1R and A3R), and potassium (K_{ATP}) and calcium channels (activated by A_1R) are also regulated by adenosine (16) (Fig. 2).

Among the numerous physiological responses regulated by adenosine receptors, epithelial ion and fluid transport, tissue barrier maintenance, hypoxia, ischemic preconditioning, and inflammation have been closely linked to CD73 activity (29). Many studies have used in vivo administration of the active soluble CD73 enzyme to demonstrate adenosine-dependent CD73 functions. However, the possibility that the soluble enzyme could have a nonenzymatic role cannot be ruled out without side-by-side testing of active soluble CD73 enzyme and a catalytically inactive soluble version. Based on the CD73 crystal structure, substrate binding is highly dependent on hydrophobic interactions with the side chains of residues F417 and F500 (58). Therefore, administration of soluble CD73 enzyme containing inactivating mutations at one or both of these sites could serve as an appropriate control to separate enzymatic from nonenzymatic effects in reconstitution studies.

While the AMPase activity defines CD73 function under many settings (8, 10, 46, 97, 107), it is not exclusive. For example, CD73 can bind various components of the extracellular matrix (ECM), including fibronectin and laminin (75, 99, 100). These early studies used CD73 isolated from chicken gizzard and human BCS-TC2 and Rugli cells and found evidence that the interactions with these ECM components were saturable and specific. A later study using human CD73 did not find evidence to support such an interaction but reported that CD73 bound to the ECM glycoprotein tenascin-C and that the adhesion and migration of MDA-MB-231 breast cancer cells could be controlled by this interaction (90). Thus it appears that CD73 binding to ECM components in vitro is highly context-specific and warrants further investigation into the in vivo relevance of this function.

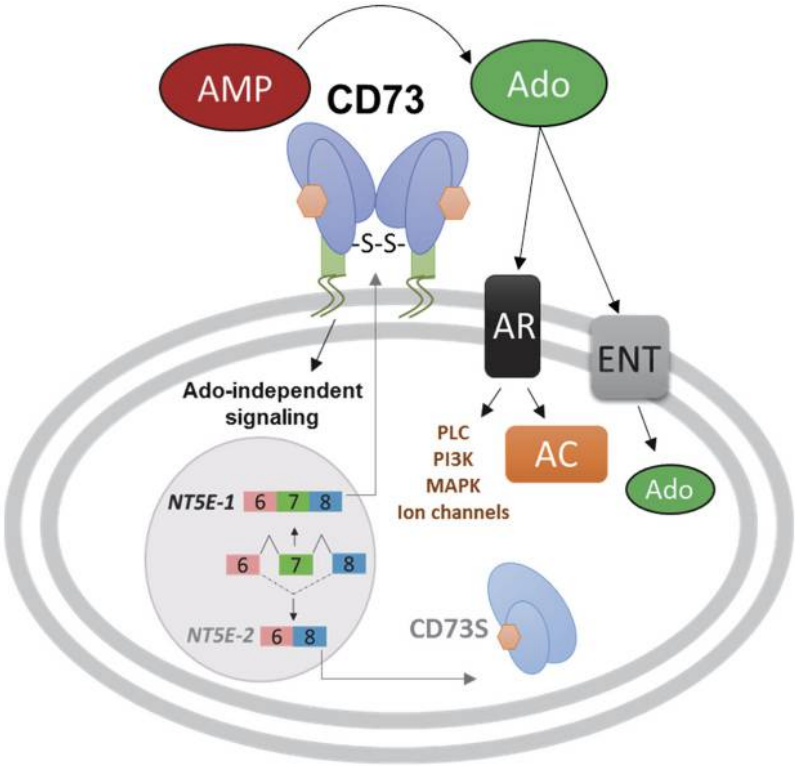

Fig. 2. Cluster of differentiation 73 (CD73) molecular and functional properties. CD73 is an ectoenzyme that converts adenosine monophosphate (AMP) to adenosine (Ado). Extracellular adenosine can activate 4 types of G protein-coupled adenosine receptors (ARs): A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R. Adenylate cyclase (AC) is inhibited by A_1R/A_3R and activated by $A_{2A}R/A_{2B}R$. ARs can also signal through phospholipase C (PLC), phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and ion channels. Alternatively, adenosine can be taken up intracellularly via equilibrative nucleoside transporters (ENT). Adenosine-independent CD73 signaling has been reported to occur via its glycosylphosphatidylinositol anchor, protein-protein interactions, and other mechanisms. The active CD73 enzyme is a disulfide-bonded (S-S) homodimer. CD73 undergoes *N*-linked glycosylation (orange hexagons). A less abundant, shorter intracellular splice variant isoform (CD73S) is produced by alternative splicing (exon 7 skipping) of the human *NT5E* gene (*NT5E-2*).

Several studies have revealed important functions of CD73 that are independent of its activity as an AMPase, including *1*) activating T cells by acting as a costimulatory signaling molecule (86), *2*) facilitating lymphocyte attachment to the endothelium (2) inducing integrin clustering (3), *3*) rendering leukemic cells resistant to apoptosis via GPI-anchor-dependent mechanisms (68), *4*) inducing phosphorylation of endothelial and lymphocyte proteins in response to antibody ligation (4, 34), and *5*) inhibiting metastasis of breast cancer cells upon membrane clustering and internalization (107). Furthermore, CD73 function and regulation are known to differ between cell types with respect to phospholipase sensitivity, shedding from the cell membrane, and ability to trigger intracellular signals in response to antibody stimulation (4). It is not known whether these observations are linked to a common nonenzymatic function of CD73, such as the intriguing possibility that CD73 may also "moonlight" as a receptor for a putative endogenous ligand. Although this idea was proposed over 20 years ago (86), it remains an open question.

CD73 in Mammalian Physiology and Human Disease

The *Nt5e*$^{-/-}$ mice that were generated and first described by Thompson and colleagues 15 years ago (108) have been used in numerous studies to uncover a number of phenotypes (Fig. 3). Importantly, most of these phenotypes are not present under baseline conditions but are unmasked when the mice are subjected to various challenges, as described below. In 2011, work from the National Institutes of Health Undiagnosed Diseases Program identified *NT5E* missense mutations leading to catalytically compromised CD73 function in three families afflicted with symptomatic arterial and joint calcifications (CALJA; OMIM 211800) (52, 98). The exact mechanisms for how these mutations contribute to the pathogenesis of the disease, referred to as "arterial calcifications due to deficiency of CD73," have not been elucidated, in part, because in vivo mouse models do not recapitulate the major phenotypes of the human disease (53).

One major difference between *NT5E* in humans and other species is that humans express several transcript variants as a result of alternative splicing. There is direct evidence for reciprocal regulation between the *NT5E-1* transcript (NM_002526), which encodes canonical CD73, and *NT5E-2* (NM_001204813), which encodes a shorter CD73 (CD73S) polypeptide (94). Under baseline conditions, *NT5E-2* is expressed at low levels across most human tissues, but both *NT5E-2* and its product CD73S are upregulated in liver cirrhosis and cancer (94). Compared with canonical CD73, CD73S lacks 50 amino acids in the COOH-terminal catalytic/dimerization domain, leading to loss of dimerization and enzymatic activity. Furthermore, in vitro overexpressed CD73S interacts with and promotes the proteasomal degradation of canonical CD73, thus acting in a dominant-negative fashion (94). In light of the species differences in CD73 regulation and associated disease phenotypes, it will be critical for future studies to integrate findings from in vivo studies on the *Nt5e*$^{-/-}$ mice (with and without appropriate stress challenges) with human-derived models, such as primary tissues, induced pluripotent stem cells (iPSCs), or tissue organoids. This will open new avenues to explore CD73 biology and disease mechanisms.

CD73 FUNCTIONS IN THE CNS

Multiple studies have implicated CD73 in CNS functions, including locomotion and behavior (9, 61), memory and plasticity (14, 125), sleep regulation (123), thermoregulation (73),

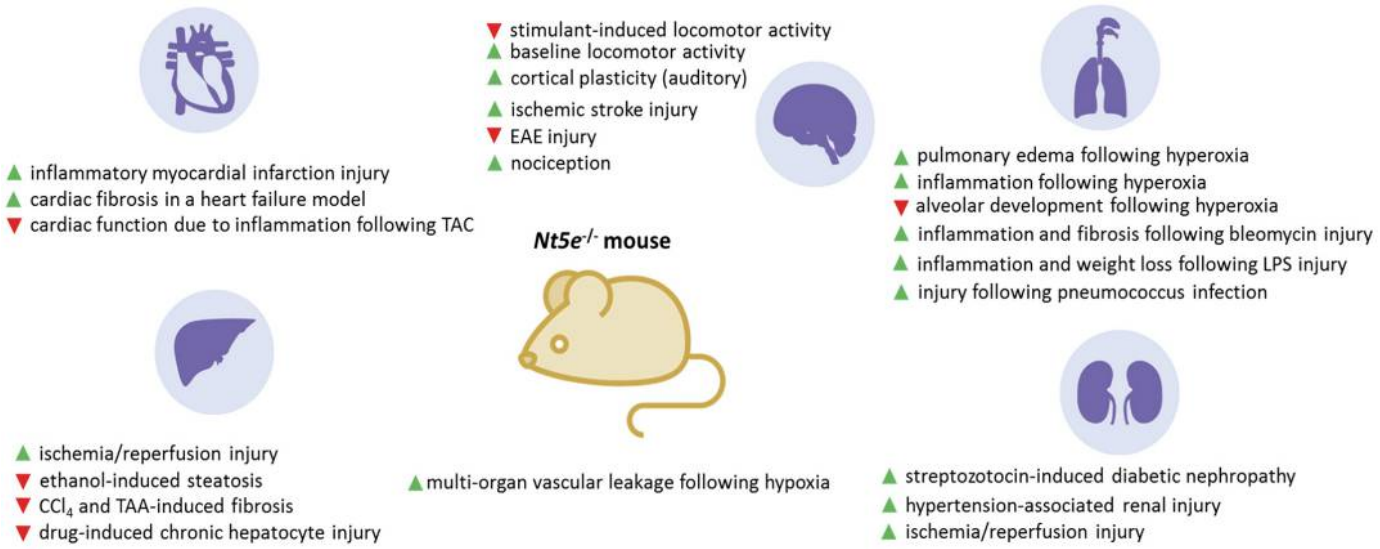

Fig. 3. Tissue-specific functions of cluster of differentiation 73 (CD73) demonstrated in studies using *Nt5e*$^{-/-}$ mice. The initial characterization of whole body CD73 knockout (*Nt5e*$^{-/-}$) mice showed vascular leakage in multiple tissues in response to normobaric hypoxia. Subsequent studies revealed multiple tissue-specific phenotypes that are either exacerbated [green (up) arrowheads] or attenuated [red (down) arrowheads] in the *Nt5e*$^{-/-}$ compared with wild-type mice. The majority of studies utilized the global knockout, and a few (see text) used tissue-specific knockouts. EAE, experimental autoimmune encephalomyelitis; TAA, thioacetamide; TAC, transverse aortic constriction.

host-pathogen interactions during brain infection (66), inflammation (69, 82, 115), and nociception. Below, we highlight several studies describing both novel and well-established mechanisms of CD73 in the brain and spinal cord.

CD73 Expression and Distribution in the CNS

Immunohistochemical localization of CD73 in mouse brain in two independent studies revealed intense specific staining in the striatum (9, 61), globus pallidus, choroid plexus, and meninges (61). Biochemically, CD73 contributes ~90% of the 5′-NT activity across the brain (61). The distribution of CD73 in the mouse spinal cord was characterized with the use of specific neuronal subtype markers, showing strong expression on L_3–L_5 dorsal root ganglion neuron membranes, particularly on the subset of neurons that express nociceptive neuron markers (96). Axon terminals in the lamina II of the spinal cord express CD73, along with another ectonucleotidase, prostatic acid phosphatase (PAP). Similar to CD73, PAP hydrolyzes AMP to produce adenosine, and both proteins and their corresponding activities decrease in response to nerve injury (96). Since the activities of CD73 and PAP enzymes are sensitive to pH (96), it was proposed that functional predominance of one enzyme may be relevant under certain conditions, such as during inflammation or tissue acidosis (96). CD73 is also abundantly expressed on astrocytes and may control their functions, such as migration and responses to injury (1, 20, 121).

CD73 Controls Locomotion

In the striatum, CD73 is closely associated with the $A_{2A}R$ in the postsynaptic compartment. This close interaction appears to be important for controlling locomotion, because locomotor activity is decreased in *Nt5e*$^{-/-}$ compared with wild-type (WT) mice after repeated amphetamine administration, similar to $A_{2A}R$ knockout mice (9). In contrast, baseline locomotion in the *Nt5e*$^{-/-}$ mice is increased when measured in the elevated-plus-maze, open-field, and circadian activity tests and monitored in the housing cages (61). Therefore, the effect of CD73 on locomotion appears to be context-specific and likely subject to the spatiotemporal dynamics of the signaling pathways over which CD73 exerts control, together with other ectonucleotidases and specific adenosine receptor subtypes.

Thalamic CD73 Inhibits Auditory Cortex Plasticity

The auditory cortex of adults, unlike newborns, lacks the plasticity required to tune neural circuitry upon passive exposure to auditory inputs from the environment (55). Recently, Blundon et al. (14) identified CD73-generated adenosine and subsequent A_1R activation to be a key mechanism for age-dependent decline in auditory cortex plasticity. Genetic deletion of the A_1R from the auditory thalamus of mature mice promoted plasticity of the auditory cortex after passive tone exposure (14). Compared with neonates, thalamic expression of CD73 in mature mice was significantly elevated, which paralleled increased adenosine production (14). In mature *Nt5e*$^{-/-}$ mice exposed to a pure tone, auditory cortex plasticity was induced, and frequencies were better distinguished in tone-exposed than tone-naive *Nt5e*$^{-/-}$ mice (14). These results have potential implications for restoring cortical plasticity via CD73 inhibition in learning and other contexts, such as recovery after stroke.

CD73 Mediates CNS Inflammation

Given the central role of adenosine as an immunomodulator, several studies have addressed the function of CD73 in brain inflammation (69, 82, 115). Using genetic and pharmacological approaches, Petrovic-Djergovic and colleagues demonstrated a protective role of CD73 in neuroinflammation due to ischemic stroke (82). Specifically, *Nt5e*$^{-/-}$ mice were more susceptible to ischemic stroke injury, and influx and activation of macro-

phages and proinflammatory markers, such as IL-1β, IL-6, and TNF-α, were increased in ischemic tissue (82). This effect was reversed by administration of soluble CD73, suggesting that the effect was adenosine-mediated (82).

Furthermore, bone marrow transplantation experiments demonstrated that the protective effect of CD73 stemmed from tissue-resident cells, as opposed to CD73 on circulating immune cells that infiltrated after the injury (82). While the specific role of CD73 on astrocytes has not been examined, it is possible that astrocytes play a key role in this model, since astrocytes contribute CD73-generated adenosine during inflammatory states (28, 121) and control neuronal injury following ischemic stroke (105). Similar to the brain, protective effects of CD73 during ischemic tissue injury have also been reported in the heart, liver, and kidneys, as shown in Fig. 4 and discussed below.

In contrast to the stroke model, CD73 was found to be proinflammatory in a mouse model of experimental autoimmune encephalomyelitis (EAE), which mimics inflammation associated with multiple sclerosis (69). Whereas WT mice displayed a weak tail and partial hindlimb paralysis by 3 wk of disease onset, the *Nt5e*$^{-/-}$ mice only had a weak tail, and the disease did not worsen over time (69). Lymphocyte infiltration into the brain was significantly blunted in the *Nt5e*$^{-/-}$ mice, implicating CD73 as a facilitator for the entry of pathogenic T cells into the CNS (69). Similar to the stroke model, adoptive transfer studies demonstrated a role for CD73 in nonhematopoietic cells, potentially choroid plexus epithelial cells, which expressed CD73 in the WT mice (69). Modulation of blood-brain barrier function via CD73-generated adenosine and activation of A_1R and $A_{2A}R$ is one potential mechanism behind the increased lymphocyte infiltration and inflammation in the EAE model (23).

In combination, these studies demonstrate that CD73 can exert pro- or anti-inflammatory effects in the brain, depending on the specific inflammatory condition and the cell types involved, which is an important consideration for potential therapeutic applications of CD73 modulators in CNS inflammation.

Neuronal CD73 Regulates Nociception

CD73 and two additional nucleotidases [PAP and tissue-nonspecific alkaline phosphatase (TNAP)] generate extracellular adenosine in the spinal cord to regulate the function of pain-sensing neurons (101, 102). A series of studies utilizing supplementation with soluble mouse CD73 enzyme in *Nt5e*$^{-/-}$ mice demonstrated that CD73 plays a key role in regulating nociception in the mouse spinal cord (97, 115). Intrathecal administration of soluble mouse CD73 protein elicited dose-dependent and long-lasting (2 days) antinociceptive effects in response to heat-induced pain (97). Similarly, soluble CD73 had antinociceptive effects in inflammatory and neuropathic pain models (97). At the molecular level, these effects were the result of A_1R activation, since soluble CD73 did not produce antinociceptive effects in $A_1R^{-/-}$ mice (97). The relative contribution of CD73 on hematopoietic cells vs. neurons in the inflammatory pain models is not clear, because studies using bone marrow chimera have not been performed. However, upon spinal cord injury, CD73 promotes polarization of macrophages and microglia to the M2 anti-inflammatory phenotype, suggesting that immune cells may play at least a partial role in the protective mechanism of CD73 in pain models (115).

CD73 FUNCTIONS IN THE HEART

A brief historical account of some of the studies (40, 59) leading to the recognition of CD73 as a key player in the cardiovascular system was provided by Olsson in 2004 (76). Other relevant work on purinergic signaling in the heart was highlighted in a review by Burnstock and Pelleg (21). Here, we focus on the most recent work regarding cell type-specific functions of CD73 in the cardiovascular system and its protective functions in myocardial infarction (MI) and heart failure.

CD73 Expression and Distribution in the Heart

In the cardiovascular system, CD73 expression was detected on smooth muscle cells (116), endothelial cells (74, 84), and resident lymphocytes (15). However, there is a discrepancy in some of the published studies regarding CD73 expression on cardiomyocytes, smooth muscle cells, and endothelial cells in normal mouse heart. Whereas one study reported the absence of CD73 from these cell types in the normal mouse heart (15), other studies in mice (37, 43), as well as data from the Human Protein Atlas, report moderate CD73 protein expression in these cell types under basal conditions (110). The reason for

				
Experimental model	Ischemic stroke	Myocardial infarction	Ischemia/reperfusion	Ischemia/reperfusion
Cell types or mechanisms promoting injury	• Macrophages • Neutrophils • Resident microglia	• Fibroblasts • Granulocytes • Antigen-presenting cells	Complement pathway	• Neutrophils • Macrophages
Cell types involved in tissue protection from injury	Tissue-resident cells	Cardiac T-cells	Not investigated	• Proximal tubular epithelial cells • Cortical fibroblasts

Fig. 4. Cluster of differentiation 73 (CD73) confers protection against ischemia-reperfusion injury in multiple tissues. In vivo studies using CD73 knockout mice have shown that CD73-generated adenosine is protective in ischemia-reperfusion injury of brain, heart, liver, and kidney. While in all cases CD73-generated adenosine is required for the protection, different cell types participate in the different tissues.

this discrepancy could be that the disruption of tissue architecture during digestion and processing for cell sorting activated mechanical signaling, which triggered downregulation of surface CD73 from nonimmune cells in the heart tissue (15). One potential molecular mechanism for CD73 downregulation during tissue digestion may be the actions of the mechanosensitive cytoskeletal protein kindlin-2, a modulator of integrin signaling in endothelial cells and cardiomyocytes (35) that regulates CD73 trafficking to the membrane (84). Therefore, assessing expression and function of CD73 on cells isolated from the heart and other solid tissues requires careful consideration of the experimental conditions used to dissociate the cells.

CD73 Protects in MI Injury

Myocardial injury during acute MI is the result of ischemia-reperfusion (I/R) injury. Healing and recovery of tissue function following MI is dependent on T cell-expressed CD73, which decreases inflammation through the generation of adenosine (17). Specifically, circulating T cells invade the injured heart after infarction and upregulate expression of hydrolyzing enzymes that act on ATP, cAMP, and NAD, culminating in adenosine production via CD73 (17). Activation of $A_{2A}R$ and $A_{2B}R$, which signal through G_s proteins, leads to reduction in the release of inflammatory mediators. T cells from *Nt5e*$^{-/-}$ mice are skewed toward Th1 and Th17 types, resulting in increased levels of their respective proinflammatory cytokine products IFN-γ and IL-17 (17). Along those lines, monocytes cocultured with mesenchymal stem cells upregulate CD73 expression in vitro and in vivo in post-MI swine heart (70), which promotes an anti-inflammatory state and implicates CD73 in the healing functions of mesenchymal stem cells.

Importantly, the anti-inflammatory properties of CD73 that have been reported in animal models of myocardial injury hold true in human patients following cardiac arrest (CA), which results in global I/R injury (89). Higher numbers of CD73$^+$ lymphocytes were associated with improved survival after CA, potentially via anti-inflammatory actions. Specifically, CD73$^+$ lymphocytes isolated from CA patients were able to inhibit the production of proinflammatory stimuli (TNF-α and reactive oxygen species) by myeloid cells in vitro (89).

Interestingly, a functionally significant upregulation of CD73 on epicardium-derived cells (EPDCs) following MI promotes the release of proinflammatory cytokines and the profibrogenic matrix protein tenascin-C (49). EPDCs, which are normally quiescent in the adult heart, are activated and give rise to multiple cell types following ischemic heart injury. Unlike the responses in T cells, increased production of CD73-generated adenosine and $A_{2B}R$ activation stimulate the release of proinflammatory cytokines (IL-6, IL-11, and VEGF) from EPDCs (49). These studies reveal that CD73 actions during and after MI are orchestrated by multiple cell types.

CD73 Protects in Experimental Heart Failure

In a mouse model of heart failure induced by transverse aortic constriction (TAC), CD73 expression is upregulated across multiple T cell populations (cytotoxic, helper, and regulatory) in a time-dependent manner (85). This upregulation appears to serve a protective role, since mice lacking *Nt5e* globally, or only on T cells, exhibit increased fibrosis and significant impairments in cardiac function after TAC (85). This, in turn, coincides with increased secretion of proinflammatory cytokines (IL-3, IL-6, and IL-13) from T cells devoid of CD73. Thus, similar to the findings in the MI model, CD73 on T cells limits excessive inflammation during TAC-induced cardiac injury. These studies highlight the need for careful consideration of the cardioprotective effects of CD73 in situations where systemic anti-CD73 therapy is being considered for noncardiac indications, such as cancer.

CD73 FUNCTIONS IN THE LUNG

Adenosine is a key regulator of respiratory function, and adenosine receptor signaling controls homeostasis of airway epithelial cells, protects tissue barrier function via activity on endothelial cells, and regulates secretion of inflammatory mediators from a number of immune cell types (122). One of the earliest phenotypes reported using the global *Nt5e*$^{-/-}$ mouse was vascular leakage in response to normobaric hypoxia in multiple tissues, but this was most pronounced in the lung (108). Aside from being a key regulator of lung injury in response to changes in oxygen, recent studies highlight CD73 functions in lung inflammation and fibrosis.

CD73 Expression and Distribution in the Lung

Together with TNAP, CD73 is the major regulator of adenosine production on airway surfaces (83). The Human Protein Atlas reports high CD73 expression on human pneumocytes, which exhibit both cytoplasmic and membrane distribution (110). CD73 activity contributes to the majority of extracellular adenosine production on mucosal and serosal surfaces of human airway epithelia (83). Extracellular adenosine, in turn, controls proper cilia beating frequency and ion transport (63, 71). In addition to this physiological role, CD73-generated adenosine by epithelial cells is protective during acute lung injury by reducing endothelial permeability (36). Therefore, CD73 activity on airway epithelial cells regulates mucociliary clearance and aids in protection against infectious and noninfectious lung diseases (Fig. 5).

CD73 Maintains Tissue Barrier Function in Hypoxia

Hypoxia-induced vascular leakage in *Nt5e*$^{-/-}$ mice is only partially reversed by adenosine receptor agonists and administration of soluble CD73, leaving open the possibility for additional nonenzymatic functions of CD73 that may be compromised in the *Nt5e*$^{-/-}$ mouse (108). Subsequent work showed that CD73 is a necessary target of IFN-β-mediated protection against vascular leakage in the lungs (57) and was used as a rationale for an open-label clinical trial examining the effectiveness of intravenous IFN-β_1 on acute respiratory distress syndrome mortality (11). The latter study demonstrated that CD73 was significantly upregulated ex vivo in peribronchiolar vessels of cultured human lung tissue in response to IFN-β_1 treatment and that IFN-β_1 attenuated 28-day mortality (8% mortality in treated patients vs. 32% mortality in the control untreated group) (11). Although it was targeted via an indirect, cytokine-dependent mechanism, these findings underscore the translational potential of CD73-mediated protection during hypoxic lung injury.

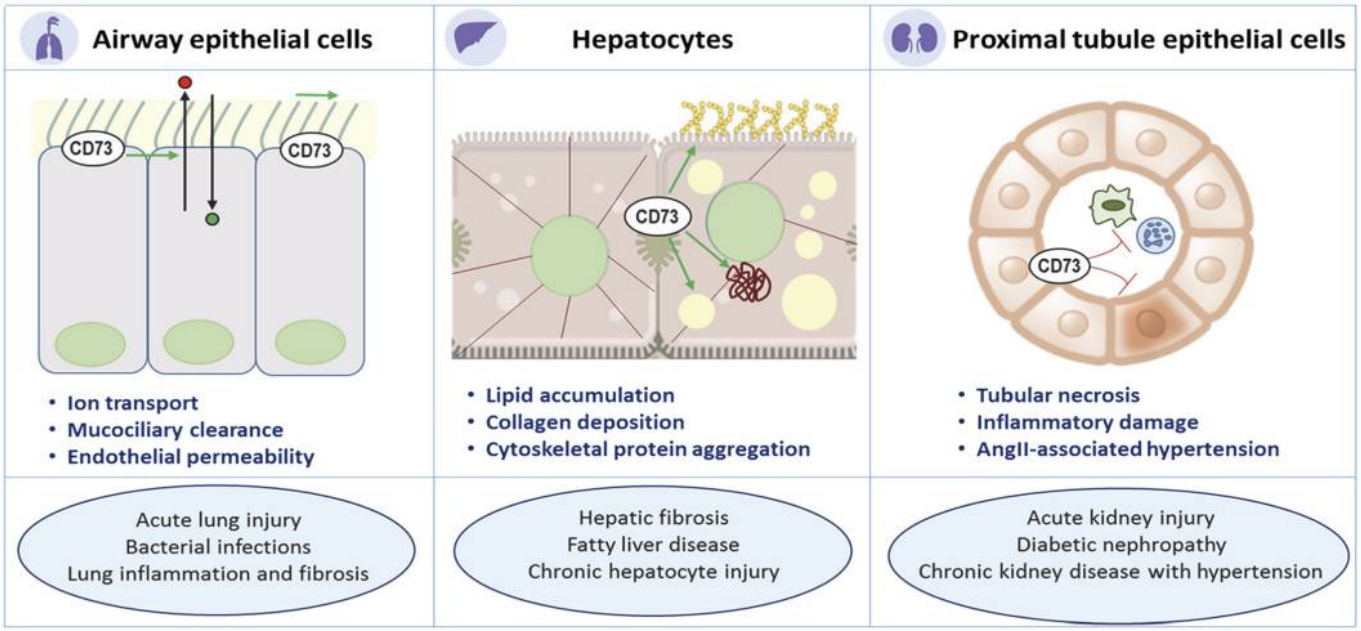

Fig. 5. Cluster of differentiation 73 (CD73) mediates homeostatic and injury responses in epithelial cells to control respiratory, hepatic, and renal functions. In the respiratory system, CD73 is the major source of extracellular adenosine on airway epithelial surfaces. Extracellular adenosine regulates mucociliary clearance and ion (e.g., chloride) exchange and preserves tissue barrier function by reducing endothelial permeability. In the liver, CD73 is primarily expressed on the apical (bile canalicular) membrane of hepatocytes. In vivo studies using multiple injury models have shown decreased steatosis, fibrosis, and chronic stress-associated protein aggregation in hepatocytes from mice lacking CD73 globally, suggesting that CD73 is a promoting factor in this setting, although the cell-intrinsic vs. cell-extrinsic mechanisms have not been resolved. In proximal tubular epithelial cells of the kidney, CD73 blocks inflammatory damage and necrosis during renal ischemia-reperfusion injury by activating adenosine A_{2A} receptors (A_{2A}Rs) on neutrophils and macrophages. CD73, via its epithelial and endothelial functions, is protective in the setting of diabetic nephropathy. In chronic kidney disease, CD73 promotes hypertension in an endothelin-1- and A_{2B}R-dependent manner. AngII, angiotensin II.

CD73 Protects Against Alveolar Damage in Hyperoxia

CD73 is also highly upregulated in response to hyperoxia, and *Nt5e*$^{-/-}$ mice develop more severe pulmonary edema during hyperoxic lung injury. The latter effect, which is also phenocopied in $A_{2A}R^{-/-}$ mice, is attributed to loss of barrier function in the setting of decreased adenosine production (30). In a newborn mouse model of hyperoxia, elevation of CD73-generated adenosine is necessary to minimize abnormal alveolar development (65). Specifically, exposure of newborn mice to severe hyperoxia (95% O_2) significantly impairs alveolar development, which is exacerbated in *Nt5e*$^{-/-}$ mice, and leads to 100% mortality by *day 11* of exposure (in contrast to 44% mortality in the WT mice). When compared in a less severe (70% O_2) setting, inflammation was increased in *Nt5e*$^{-/-}$ compared with WT mice, as evidenced by increased lung infiltration of macrophages and lymphocytes. In humans, hyperoxic conditions (e.g., oxygen supplementation) can promote bronchopulmonary dysplasia in premature neonates. Interestingly, caffeine, which is a nonselective adenosine receptor antagonist, reduces the rate of bronchopulmonary dysplasia in human neonates (91). The seeming contradiction between the preclinical findings in mice and humans could be due to general species differences, developmental stage (e.g., newborn mice were full-term and human neonates were premature, weighing between 500 and 1,250 g), or, possibly, an adenosine-independent protective role of CD73 that is compromised in the *Nt5e*$^{-/-}$ model. Similar to other systems, careful integration of data using animal models needs to be weighed alongside appropriate human-derived model systems to better understand which mechanisms of CD73 are conserved across species.

CD73 Has Both Protective and Promoting Effects in Lung Inflammation and Fibrosis

Adenosine is produced as a protective response in the setting of lung inflammation, and CD73 plays a key role in this mechanism, as initially shown using a bleomycin model of inflammatory lung injury (113). Bleomycin-treated *Nt5e*$^{-/-}$ mice exhibited enhanced inflammation, collagen production, and more severe lung fibrosis, which were attenuated by intranasal administration of exogenous nucleotidase. In further support of an anti-inflammatory role of CD73, *Nt5e*$^{-/-}$ mice exhibited increased weight loss and inflammation in response to intratracheal administration of lipopolysaccharide, concomitant with significant transcriptional upregulation of TNF-α, IL-1β, and IL-6 (38). These effects were attributed to diminished adenosine generation by regulatory T cells, and the inflammatory phenotype was partially rescued by administration of soluble CD73. The anti-inflammatory properties of CD73-generated adenosine also extend to pneumococcus infection, involving adenosine-mediated mobilization of polymorphonuclear leukocytes (PMNs) to control bacterial burden (18). While the early PMN response is beneficial, prolonged presence of PMNs in the lung is detrimental. Extracellular adenosine produced by CD73 was generated in response to pneumococcus infection and restricted later-stage PMN movement across the endothelium into the lungs (18). In the absence of CD73, more PMNs transmigrated but failed to control

bacterial burden. Therefore, CD73 both limits PMN migration and enhances their bactericidal activity against pneumococcus.

In contrast to the above-described anti-inflammatory and antifibrotic functions, CD73 is a promoting factor in the setting of radiation-induced lung fibrosis (114). CD73 activity was increased threefold in mice that received 15-Gy whole thorax irradiation within 25–30 wk after the treatment, at which point significant fibrosis was present. The elevated CD73 activity coincided with increased tissue infiltration of immune cells and a significant increase in adenosine concentration in the bronchoalveolar lavage fluid. However, in $CD73^{-/-}$ mice or in WT mice treated with the anti-CD73 monoclonal antibody TY/23, development of epithelial damage and fibrosis was significantly blunted in response to the same radiation treatment, despite similar numbers of infiltrating leukocytes. While the specific cellular mechanisms are not clear, it was proposed that long-term, sustained elevation of pulmonary adenosine levels promotes pathological tissue remodeling (114).

CD73 FUNCTIONS IN THE LIVER

CD73 Expression and Distribution in the Liver

In the normal liver, CD73 is expressed on the apical membrane of hepatocytes as well as endothelial cells, albeit at lower levels than in hepatocytes (67). *NT5E* is upregulated during myofibroblast differentiation of activated hepatic stellate cells in culture (41). Additionally, $CD73^{+}$ regulatory B cells are recruited to the liver in response to hepatic inflammation (6). CD73 has emerged as a critical regulator of hepatocyte responses to different forms of injury (46, 79, 80, 95), illuminating common disease mechanisms that may be exploited therapeutically.

CD73 Protects During Ischemic Preconditioning

Ischemic preconditioning (IP), which involves brief episodes of I/R, is the mechanism by which a tissue mounts a protective response to subsequent prolonged I/R injury. In mouse liver, within 90 min of initiation of IP, CD73 gene expression is strongly upregulated and protein levels significantly increase by 120–180 min (46). This induction of CD73 is critical for the protective benefit of IP, which is eliminated in $Nt5e^{-/-}$ mice and in WT mice treated with the CD73 inhibitor adenosine 5′-(α,β-methylene)diphosphate, resulting in diminished IP protection and significantly more severe I/R damage to the liver. Furthermore, administration of soluble CD73 protein attenuated I/R injury in WT mice by decreasing complement activation (46) (Fig. 4).

Although it was not determined which specific adenosine receptor type mediated these effects, a previous study implicated A_{2A}Rs in hepatic protection against hepatic I/R injury via inhibition of natural killer cells (31, 62). Another possibility is that the protective effects of CD73 in hepatic protection by IP may be mediated by the A_{2B}R, which is known to be involved in myocardial protection by IP, as demonstrated by pharmacological and genetic approaches (37).

Adenosine and CD73 Regulate Hepatic Fibrosis

Liver fibrosis is a major health problem that arises due to multiple forms of chronic liver injury, such as alcohol consumption, viruses, and metabolic disorders. There are no approved antifibrotic therapies to combat the functional deterioration of the liver. Although it was shown ~20 years ago that chronic adenosine administration can reverse CCl_4-induced liver fibrosis in male rats (48), conflicting results have been reported in studies using genetic mouse models and adenosine receptor modulators. For example, compared with WT mice, male $A_{1A}R^{-/-}$ mice have decreased fibrosis when CCl_4 is used as the fibrogenic agent and worsened fibrosis induced by bile duct ligation (118). This demonstrates that the cellular mechanisms that initiate and propagate the fibrogenic response are critical in understanding the roles of adenosine in this context.

Genetic deletion or pharmacological inhibition of the A_{2A}R in a mixed cohort of male and female mice caused increased inflammation and liver damage following concanavalin A liver injury, but in another study, $A_{2A}R^{-/-}$ mice developed less severe fibrosis in response to thioacetamide (TAA) or CCl_4 treatment (25). In agreement with the latter study, an A_{2A}R antagonist was reported to be protective against ethanol-induced exacerbation of fibrosis due to CCl_4 toxicity as the primary hit (27). In addition to the use of different profibrogenic insults and the inherent limitations of such models (32), there are other caveats to consider when reconciling the various studies and outcomes, including the possibility of sex-specific differences. For example, the CCl_4/TAA study did not specify the sex of the mice used (25), and only female mice were included in the CCl_4 + ethanol study (27). Thus, sex-specific differences are a likely important contributor for the different effects in response to A_{2A}R genetic deletion or pharmacological blockade.

In line with a potential profibrogenic role of adenosine, CD73 has also been implicated as a contributor to liver fibrosis (Fig. 5). Specifically, CCl_4-induced liver fibrosis is blunted in $Nt5e^{-/-}$ compared with WT mice by ~50% when measured histologically and by ~20% based on hydroxyproline content (80). In the TAA model of fibrosis, the differences between WT and $Nt5e^{-/-}$ mice are fairly small (fibrosis score is attenuated by 15–20% in the $Nt5e^{-/-}$ mice) (80). It is not clear whether the attenuated phenotype in the $Nt5e^{-/-}$ mice is brought on by adenosine deficiency, as no rescue-type experiment using soluble enzyme was done. In addition, it has not been determined how the induction of hepatic fibrosis affects CD73 expression, distribution pattern, and enzymatic activity in the liver (80). A subsequent study reported expression of CD73 around fibrotic nodules, but it also did not quantify protein expression levels or cell type-specific distribution (41). The cell type-specific roles of CD73 in hepatocytes vs. other cell types, such as myofibroblasts, immune cells, or endothelial cells, have not been examined in a quantitative and dynamic manner in the context of liver fibrosis. This type of information will be crucial in advancing our understanding of extracellular adenosine in liver fibrosis and may shed light on some of the contradicting results that have been published with regard to the anti- and profibrotic functions of adenosine and its receptors.

Adenosine and CD73 Regulate Hepatic Steatosis

An early phenotype of alcohol consumption is the development of hepatic steatosis, which is characterized by accumulation of lipid droplets in hepatocytes. It was reported that global deletion of the *Nt5e* gene reduced the development of

hepatic steatosis in mice in response to ethanol (79) (Fig. 5). Similar effects were seen in mice lacking A_1R or $A_{2B}R$ (79), which is interesting, given that A_1Rs ($G_{i/o}$-coupled) and $A_{2B}Rs$ (G_s-coupled) transmit different signals and exhibit a 100- to 1,000-fold difference in affinity for adenosine (16). In vitro work has demonstrated that A_1R signaling does not regulate lipogenic gene expression in hepatocytes (117), and other studies using dietary and genetic models of fatty liver have reported a protective role of adenosine signaling in fatty liver (22, 60). Specifically, genetic deletion of the $A_{2B}R$ exacerbates hepatic steatosis in ApoE-null mice fed a high-fat diet (60), and $A_{2A}R$ deletion promotes nonalcoholic fatty liver disease (NAFLD) in mice (22). These studies show that, similar to fibrosis, the effects of adenosine on hepatic steatosis may be context-dependent. It is important to note that CD73 mRNA is significantly decreased in human NAFLD biopsy specimens (95). However, hepatic mRNA and protein expression of CD73 are not always concordant, at least in mouse liver (95), and CD73 protein expression remains to be tested in human NAFLD specimens.

It is unclear whether the mild protection against ethanol-induced steatosis in the *Nt5e*$^{-/-}$ mice is mediated by adenosine or by adenosine-independent mechanisms, as in vivo enzyme reconstitution experiments have not been performed. The use of liver-specific knockout models in conjunction with more clinically representative models of fatty liver disease, such as the acute-on-chronic alcohol injury model (12) or two-hit NAFLD model (24, 111), will be necessary to assess the translational potential of CD73 in fatty liver disease development and progression.

CD73 Plays a Role in Severe Hepatocyte Injury

There is a strong genetic predisposition component to hepatocellular injury and liver disease progression in mice (45) and humans (88). Metabolomic comparison of liver injury-susceptible and -resistant mouse strains revealed CD73 to be an important player in hepatocyte injury characterized by ballooning degeneration and formation of cytoplasmic protein aggregates (Fig. 5) (95). These aggregates, called Mallory-Denk bodies (MDBs), incorporate cytoskeletal elements and stress-activated proteins and are commonly seen in hepatocytes from patients with chronic liver disease (119). *Nt5e*$^{-/-}$ mice are protected against MDB-associated hepatocellular injury induced via chronic treatment with the drug 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) (95). While the *Nt5e*$^{-/-}$ mice still develop liver injury (as indicated by elevated serum liver enzyme levels), they have attenuated hepatomegaly and lack any observable MDBs on histological evaluation. This effect is somewhat puzzling, because cell-surface CD73 enzymatic activity and protein expression are dramatically downregulated in WT mice subjected to this injury model (95). However, CD73 accumulates intracellularly in hepatocytes exposed to DDC, which leaves open the possibility that the MDB-promoting effects of CD73, as suggested by the *Nt5e*$^{-/-}$ model, are not necessarily tied to its function as an ecto-AMPase.

MDBs are observed in human hepatocellular carcinoma (HCC), the most common form of primary liver cancer (33). Interestingly, in human HCC, there is cytoplasmic accumulation and loss of plasma membrane-associated CD73 in tumor and adjacent nontumor tissue (5). However, the effect is more pronounced in tumor cells, where site-specific CD73 *N*-glycosylation is significantly altered and accompanied by decreased enzymatic activity compared with normal liver and nontumor adjacent liver from HCC patients. Specifically, tumor-associated CD73 is deficient in hybrid and complex glycans on residues N311 and N333, located in the COOH-terminal catalytic domain. Blocking N311/N333 glycosylation via site-directed mutagenesis produced CD73 with significantly decreased 5′-NT activity in vitro, similar to the primary HCC tumors. Therefore, identifying molecular regulators of CD73 glycosylation and understanding the functional outcomes of altered glycan signatures on CD73 may ultimately help resolve some of the mechanisms behind its reported adenosine-independent, nonenzymatic activities (Fig. 2).

CD73 FUNCTIONS IN THE KIDNEY

CD73 Expression and Distribution in the Kidney

Renovascular function is under the control of adenosine produced by CD73, in addition to TNAP (51). The enzymatic activity of CD73 in the kidneys is the highest of all the tissues in the body (29, 108). In rat and mouse kidneys, enzyme histochemistry and immunostaining analyses have revealed expression of CD73 on the luminal membranes of proximal tubular cells, the peritubular space, intercalated cells of the distal nephron, and cortical fibroblasts (93, 112). CD73 has mixed protective and injury-promoting functions in the kidney, depending on the type of kidney injury.

CD73 Protects in Renal I/R Injury

I/R is a leading cause of acute kidney injury, and CD73 plays a key protective role in this setting. The most severely affected region of the kidney during I/R is the lower cortex and outer medulla, where CD73 expression is also the highest (64). CD73 expressed on proximal tubular epithelial cells is a critical mediator of the protective response, which was shown using mechanistic in vivo experiments with *Nt5e*$^{-/-}$ mice and mice with a targeted deletion of CD73 from the proximal tubule compartment (PEPCK^{Cre}CD73$^{f/f}$) (103). This study showed less severe renal injury in the PEPCK^{Cre}CD73$^{f/f}$ than *Nt5e*$^{-/-}$ mice, which was attributed to a residual protective effect from CD73 expressed on cortical fibroblasts in the former model. Indeed, targeted deletion of CD73 from cortical fibroblasts using a *Foxd1*-driven Cre promoter resulted in an increased time-dependent injury response (103).

Therefore, CD73 on proximal tubular epithelial cells and cortical fibroblasts confers a protective function during I/R injury. In contrast, there was no significant difference in renal I/R injury between control mice and mice with a targeted deletion of CD73 from dendritic cells or macrophages. Reconstitution of 5′-NT activity in vivo reversed injury in the *Nt5e*$^{-/-}$ and PEPCK^{Cre}CD73$^{f/f}$ mouse models. The localized production of adenosine via CD73 was a key component at the site of injury, because it targeted the $A_{2A}R$ on neutrophils and macrophages, as shown in detail using bone marrow transplantation experiments (103).

These results align well with previous findings (56) related to the role of CD73 in perioperative ischemic acute kidney injury (56). Specifically, CD73 on proximal tubule cells was

strongly induced in response to clinically relevant concentrations of the volatile anesthetic isoflurane via a TGF-β1-dependent mechanism (56). Furthermore, isoflurane protected against renal tubular necrosis and inflammation following I/R injury in a TGF-β1/CD73/A_{2A}R-dependent mechanism (56). Together, these studies highlight, at a mechanistic level, the translational importance of CD73 in the protection against acute kidney injury. As highlighted above and summarized in Fig. 4, the protective role of CD73 during I/R injury is common across brain, heart, kidney, and liver tissues, while the cellular mechanisms mediating the protective responses are tissue-specific.

CD73 Protects in Diabetic Nephropathy

In addition to acute kidney injury, CD73 also plays a protective role in diabetic nephropathy, a chronic kidney disease that can develop in patients with long-standing diabetes mellitus. In mice, chronic administration of the chemotherapeutic agent streptozotocin (STZ) leads to selective destruction of insulin-producing pancreatic β cells, resulting in a diabetic state and nephrotoxicity, modeling aspects of diabetic nephropathy. Renal adenosine content was increased twofold in WT mice but unchanged in *Nt5e*$^{-/-}$ mice treated with a 16-wk course of STZ (104). *Nt5e*$^{-/-}$ mice exhibited signs of more severe renal dysfunction, with increases in drinking volume, urine output, glomerular filtration rate, and albuminuria. Similar effects were seen in mice lacking endothelial A_{2B}R (104). Administration of soluble CD73 restored renal adenosine content to WT levels and reversed all parameters of renal injury. Therefore, the regulated production of extracellular adenosine via CD73 and subsequent activation of the endothelial A_{2B}R are key protective mechanisms in a mouse model of diabetic nephropathy (104). Increased CD73 activity appears to be an early event in STZ-mediated renal injury, as shown using a rat model (77), and, similar to mice, adenosine administration is protective in the rat (106).

In addition to the protective effects of adenosine, CD73 induction and subsequent buffering of extracellular adenosine-ATP levels may be a mechanism to dampen the proinflammatory actions of extracellular ATP. The anti-inflammatory actions are evidenced in studies showing that *Nt5e*$^{-/-}$ mice exhibit spontaneous proteinuria and renal functional deterioration as they age because of an autoimmune inflammation affecting the glomerular endothelium (13).

CD73 Promotes Renal Injury with Hypertension

CD73 mRNA, protein, and activity were found to be elevated two- to threefold in response to angiotensin II (ANG II), which correlates with a fourfold increase in renal adenosine content (120). The increase in ANG II-stimulated renal adenosine production was CD73-dependent, as this effect is eliminated in *Nt5e*$^{-/-}$ mice. Increased systolic blood pressure following ANG II infusion is seen in WT, but not *Nt5e*$^{-/-}$, mice. This effect is mediated by the A_{2B}R, as blood pressure is not elevated in ANG II-infused mice lacking the A_{2B}R. It was proposed that a positive-feedback loop between CD73 and the A_{2B}R with hypoxia-inducible factor-2α promotes endothelin-1 expression to promote hypertension and renal injury (120). However, many of the experiments in vivo were based on correlating differences in expression levels (120). Nevertheless, CD73 protein levels (assessed by immunohistochemical staining) are two- to threefold higher in patients with chronic kidney disease with hypertension than in patients with mild disease and no hypertension and sevenfold higher than in control patients. Therefore, it would be important for future studies to assess the cell type-specific effects of CD73 in renal injury in the setting of hypertension.

CONCLUSIONS

As one of the key regulators in the purinergic signaling pathway, CD73 is central to many physiological functions. However, its importance is especially apparent in the context of acute and chronic types of injury, where CD73 activity is vital for maintaining tissue integrity and facilitating recovery. Among the key areas to be investigated over the next few years will be the cell type-specific functions of CD73 in vivo through the use of tissue-specific knockout mouse models in combination with ex vivo primary cultures. In addition, it will be important to determine which phenotypes in the animal models are reflected in human patients. The latter will involve development of new tools to study the function of CD73, such as human iPSC-derived cells and tissue organoids. It also remains to be seen whether systemic CD73-based therapy is feasible or if more targeted approaches will be necessary to avoid untoward effects. While there is overwhelming preclinical evidence to support the development of CD73-based therapies, understanding how these could impact normal physiological functions will be key.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

N.T.S. prepared figures; M.M., K.P.A., R.A.B., and N.T.S. drafted manuscript; M.M., K.P.A., R.A.B., and N.T.S. edited and revised manuscript; M.M., K.P.A., R.A.B., and N.T.S. approved final version of manuscript.

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