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REVIEW **Open Access**

Adenosine deaminase, not immune to a mechanistic rethink in central nervous system disorders?

Benjamin Hall, Jonathan G. George and Scott P. Allen

Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, UK

Summary. Adenosine deaminase (ADA) is a purine metabolism enzyme that catalyses the breakdown of adenosine and deoxyadenosine. The enzyme is important in several cellular processes, including the innate immune response and cellular differentiation, and it is also an important enzyme for the maintenance of brain homeostasis, in part due to its regulation of adenosine. Aberrant regulation of ADA enzyme activity has been linked to several neurodegenerative diseases and diseases that can result in neurological impairment. However, the mechanisms behind altered ADA regulation and how this leads to the development of neurological dysfunction are poorly characterised. This review summarises the current research on ADA and its role and regulation in disease pathology, with a focus on the central nervous system (CNS) and the neurodegenerative disease, amyotrophic lateral sclerosis (ALS).

Key words: Adenosine deaminase, Central nervous system, Pathology, Severe combined immunodeficiency, Amyotrophic lateral sclerosis

Introduction

Adenosine deaminase (ADA) is an enzyme that is vital in the maintenance of homeostasis within the body. This is evidenced by its involvement in numerous disease pathologies including those of the immune system. ADA has also long been though of as a neuromodulator (Nagy et al., 1984). Emerging evidence of its involvement in numerous disorders that affect the central nervous system (CNS) from our laboratory and others, corroborate this theory. This review will provide an overview of ADA, its regulation, and role in the body and CNS. Moreover, we will discuss the enzyme's involvement in several diseases, including those of the CNS with a focus on amyotrophic lateral sclerosis (ALS). We will discuss potential mechanisms of action for ADA's involvement in ALS and put forward recommendations for the direction of future research.

Purine Metabolism

ADA is part of purine metabolism, that includes purine de novo synthesis, purine salvage and purine degradation (Figs. 1, 2). Purine metabolism is essential in the body as it is responsible for the production of key DNA and RNA nucleotides and is therefore required for DNA synthesis (Ansoleaga et al., 2015). Moreover,

Corresponding Author: Benjamin Hall, Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, 385 Glossop Road, Sheffield S10 2HQ, UK. e-mail: bpchall1@sheffield.ac.uk

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Abbreviations. ADA, Adenosine deaminase; ADAR, adenosine deaminase acting on RNA; ADGF, ADA related growth factor; ADP, Adenosine diphosphate; ADSL, adenylosuccinate lyase; ADSS, Adenylosuccinate synthase; ALS, amyotrophic lateral sclerosis; AMP, Adenosine monophosphate; ATIC, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase; ATP, Adenosine Triphosphate; BMT, bone marrow transplant ; cAMP, cyclic adenosine monophosphate; CNS, central nervous system; DADA2, Deficiency of adenosine deaminase 2; dATP, deoxyadenosine triphosphate; DPP4, dipeptidyl peptidase-4; E2, 17Beta-oestradiol; EHNA, Erythro-9-(2-hydroxy-3-nonyl)adenine; ERK, extracellular signal-regulated kinases; ERT, enzyme replacement therapy; fALS, familial amyotrophic lateral sclerosis; FGAMS, phosphoribosylformylglycinamidine synthase; FGF2, fibroblast growth factor 2; GART, glycinamide ribonucleotide transformylase; GMP, guanosine monophosphate; HSCT, hematopoietic stem cell transplantation; IMP, inosine monophosphate; IMPDH, inosine monophosphate dehydrogenase; JNK, Jun amino-terminal kinases; MAPK, mitogen activated protein kinases pathway; mGluRs, metabotropic glutamate receptors; MN, motor neurons; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PAICS, phosphoribosyl aminoimidazole succinocarboxamide synthetase; PD, Parkinson's disease; PEG-ADA, ADA conjugated to polyethylene-glycol; PKC, protein kinase C; Poly-PR, proline-arginine poly-dipeptide repeats; PPAT, phosphoribosyl pyrophosphate aminotransferase; PRA, 5-phosphoribosyl-1-amine; PRPP, glutamine 5-phosphoribosyl-1-pyrophosphate; SAH, S-adenosylhomocysteine; SAHH, SAH Hydrolase; sALS, sporadic amyotrophic lateral sclerosis; SAM, S-adenosylmethionine; SAPK, stress activated protein kinases; SCID, severe combined immune deficiency; SNV, single nucleotide variant; T2DM, type 2 diabetes mellitus

purine metabolism produces the important metabolites adenosine and guanine that can be utilised for the generation of other metabolic intermediates. Adenosine in particular plays a crucial role in cellular energy transfer as a key constituent of adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine trisphosphate (ATP) (Ansoleaga et al., 2015). Purine bases are also used to form cofactors for enzymatic reactions, for example adenosine is a component of S-adenosylmethionine (SAM), which is formed from the combination of ATP and methionine, and is crucial for SAM-facilitated methylation of nucleic acids and metabolic intermediates (Cantoni, 1953). Purine de novo synthesis begins with the breakdown of glutamine 5-phosphoribosyl-1-pyrophosphate (PRPP) in to 5-phosphoribosyl-1-amine (PRA) and ends at the

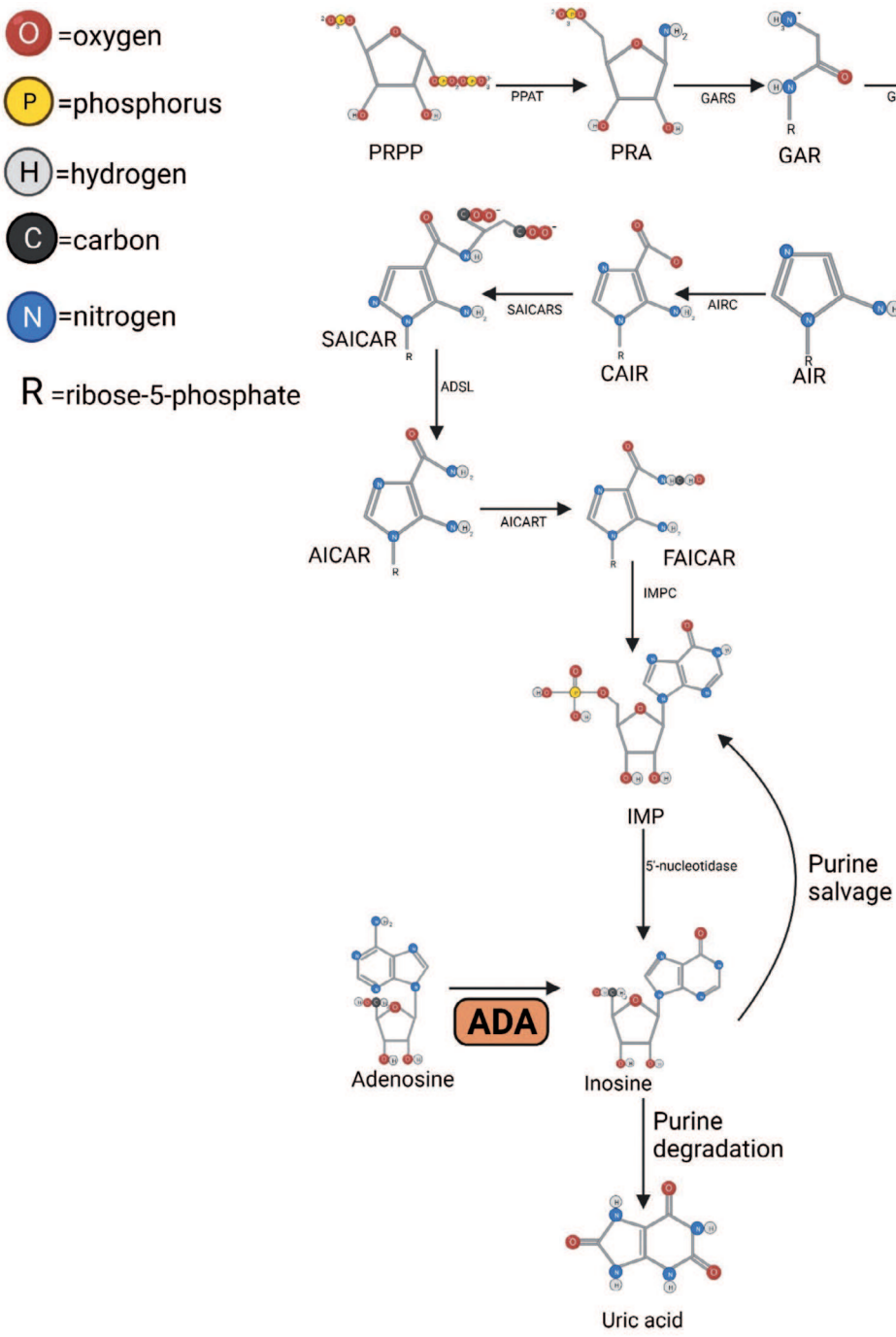

Fig. 1. Purine de novo synthesis. Purine de novo synthesis, is composed of the following intermediates: phosphoribosylpyrophosphate (PRPP); phosphoribosylamine (PRA); glycinamide ribonucleotide (GAR); N-formylGAR (FGAR); N-formylglycyamidine ribonucleotide (FGAM); aminoimidazole ribonucleotide (AIR); carboxyAIR (CAIR); succinylaminoimidazolecarboxamide ribotide (SAICAR); aminoimidazolecarboxamide ribotide (AICAR); formAICAR (FAICAR); and inosine monophosphate (IMP); and the enzymes: phosphoribosyl pyrophosphate aminotransferase (PPAT); GAR synthase (GARS); GAR transformylase (GART); FGAM synthase (FGAMS); AIR synthetase (AIRS); AIR carboxylase (AIRC); SAIRCAR synthase (SAIRCARS); adenylosuccinate lyase (ADSL); AICAR transformylase (AICART); and IMP cyclohydrolase (IMPC). IMP produced by de novo synthesis can then enter the purine salvage pathway or be broken down via the purine degradation pathway.

production of inosine monophosphate (IMP), which can then be converted into guanosine monophosphate (GMP) or AMP (Fig. 1) (Camici et al., 2018). In times of high purine demand in the cell, de novo purine enzymes can cluster to form dynamic multienzyme complexes, referred to as a 'purinosome' (for a recent review see

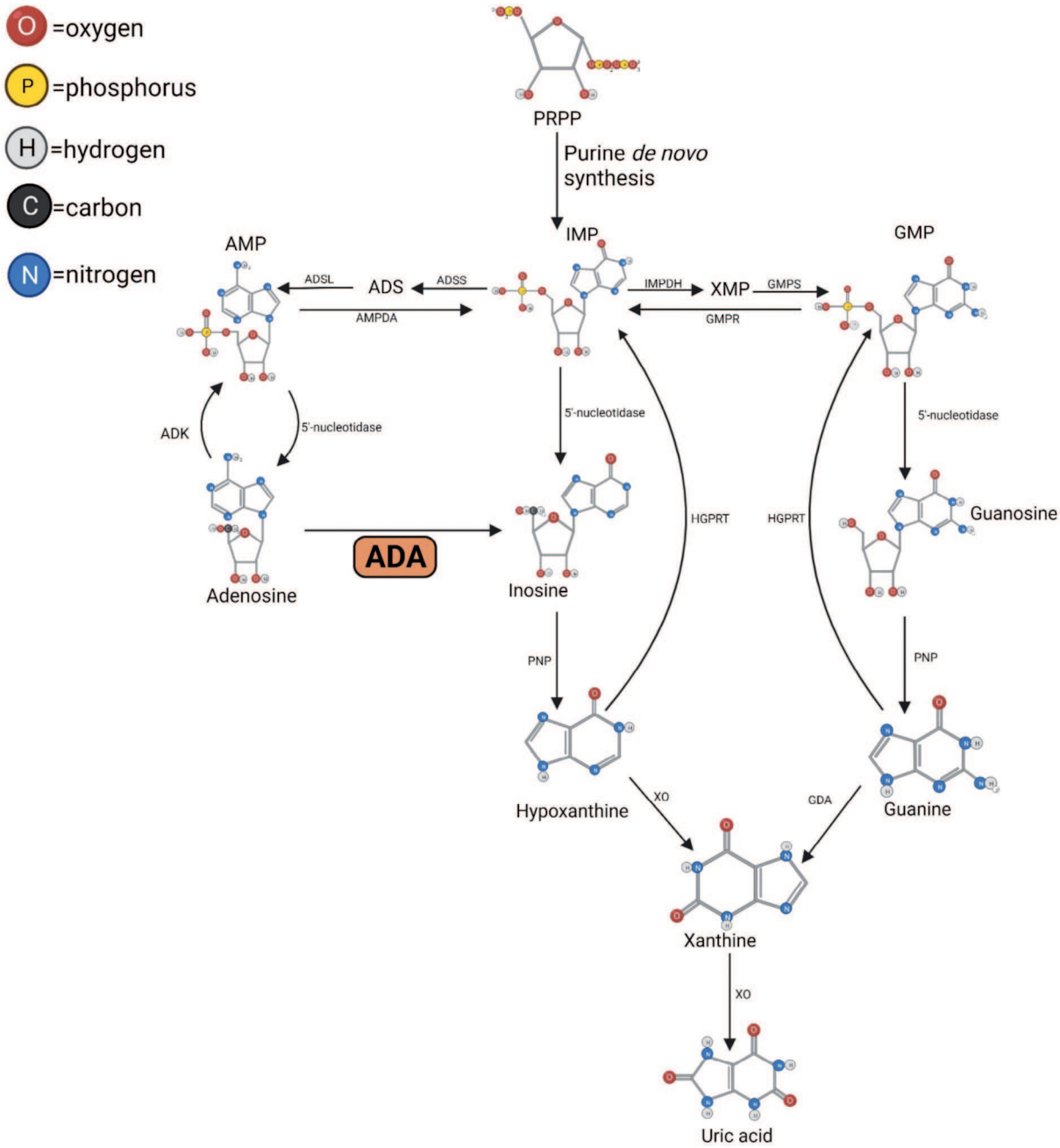

Fig. 2. Purine salvage and degradation. The purine salvage and degradation pathways involve the intermediates: adenosine monophosphate (AMP); adenylosuccinate (ADS); inosine monophosphate (IMP); xanthine monophosphate (XMP); guanosine monophosphate (GMP); adenosine; inosine; guanosine; hypoxanthine; guanine; xanthine and uric acid; and the enzymes: ADS lyase (ADSL); ADS synthase (ADSS); AMP deaminase (AMPDA); IMP dehydrogenase (IMPDH); GMP synthase (GMPS); GMP reductase (GMPR); adenosine kinase (ADK); 5'-nucleotidase; adenosine deaminase (ADA); hypoxanthine-guanine phosphoribosyl transferase (HGPRT); purine nucleoside phosphorylase (PNP); xanthine oxidase (XO) and guanine deaminase (GDA). IMP generated via purine de novo synthesis can be interconverted between AMP and GMP. AMP and IMP are both broken down to inosine and GMP is broken down into guanosine, which are further degraded to hypoxanthine and guanine respectively. Hypoxanthine and guanine can then either follow purine salvage and be reconverted to IMP or enter the purine degradation pathway and be broken down into uric acid.

Pedley and Benkovic, 2017), that co-localises with the mitochondria (Fig. 3) (Zhao et al., 2015). The purinosome consists of two parts: a core formed by the enzymes phosphoribosyl pyrophosphate amidotransferase (PPAT), 5'-Phosphoribosyl-N-formylglycinamide (GART) and phosphoribosyl formylglycinamidine synthase (FGAMS), and a group of peripheral proteins formed from phosphoribosyl aminoimidazole succinocarboxamide synthetase (PAICS), adenylosuccinate lyase (ADSL) and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (ATIC) (Deng et al. 2012). Adenylosuccinate synthase (ADSS) and inosine monophosphate dehydrogenase (IMPDH) have also been shown to associate with the purinosome (Zhao et al., 2015) (Fig. 3). Purine salvage is initiated when de novo synthesis is not possible or unable to provide the required level of nucleotides and involves the reconversion of hypoxanthine and guanine to IMP and GMP respectively, by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) (Fig. 2). Alternatively, hypoxanthine can be broken down to uric acid by the enzyme xanthine oxidase, which constitutes the purine degradation pathway (Fig. 2).

Adenosine deaminase

ADA acts as a key junction in purine metabolism, catalysing the irreversible hydrolytic deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively, substituting a molecule of ammonia for a keto group, a reaction first described in 1936 (Conway and Cooke, 1938) (Fig. 4). The importance of ADA is highlighted by its substrates, as both adenosine and deoxyadenosine are crucial for maintaining homeostasis in the body. Adenosine has important functions in energy transfer as a component of ATP and in cell signalling as a part of cyclic AMP (cAMP) alongside several other functions, modulated by its receptors. Deoxyadenosine is a base (A) in double-stranded DNA. Both molecules and their breakdown products, inosine and deoxyinosine, are also key intermediaries in purine metabolism (Fox and Kelley, 1978).

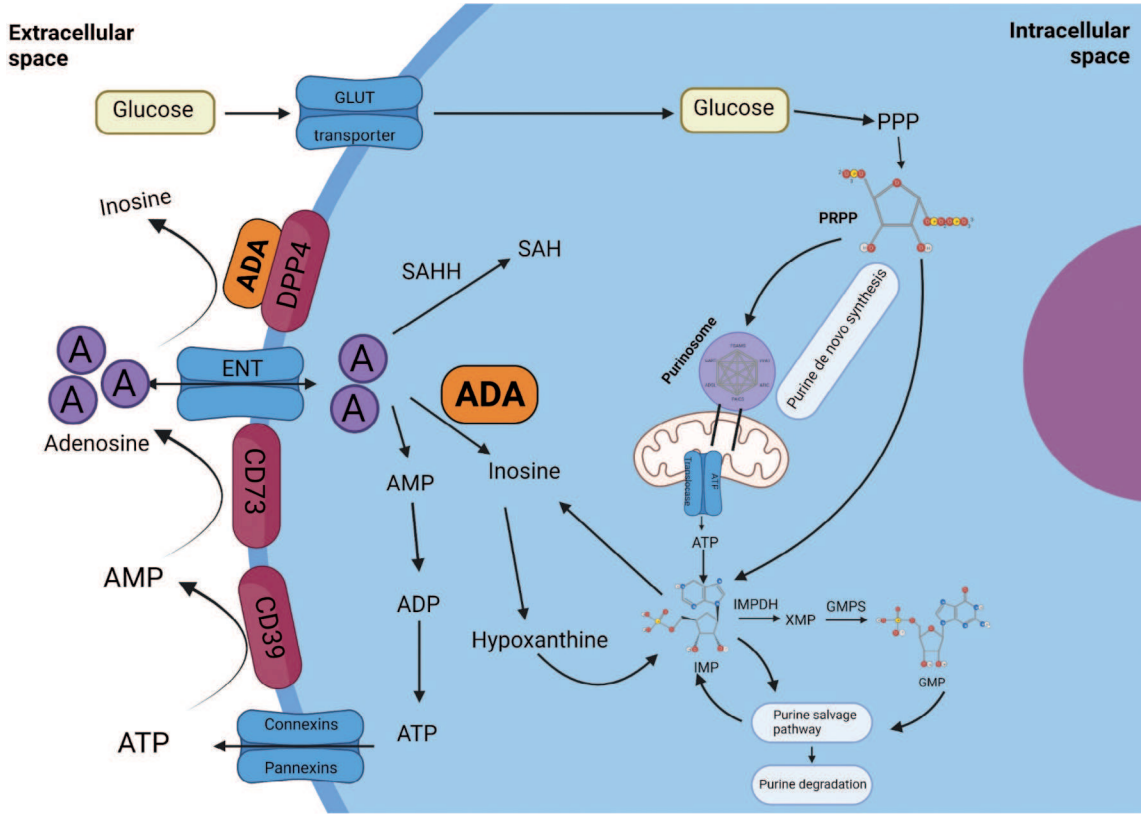

Fig. 3. Adenosine and purine metabolism in the cell. A representation of extra- and intercellular regulation of adenosine and purine metabolism. Ribose-5-phosphate, derived from glucose enters the purine de novo synthesis pathway via the purinosome which is co-localised to the mitochondria or via traditional purine synthesis to generate IMP. IMP transitions between AMP and GMP and can also enter purine salvage and purine degradation pathways. The conjunction between adenosine metabolism and purine metabolism is also represented, catalysed by the breakdown of adenosine into inosine by ADA. Extracellular ATP can be broken down by the cell surface enzymes CD39 and CD73 into adenosine, which can then be converted into inosine by ADA anchored to DPP4, re-enter the cell via equilibrated nucleoside transporters (ENTs; pictured) or can bind to adenosine receptors (not pictured). Adenosine can then be converted to inosine by ADA or combine with homocysteine to form SAH, catalysed by SAH hydrolase (SAHH).

There are two isoenzymes of ADA, ADA1 and ADA2, that are coded for by two different gene loci. The 363 amino acid ADA1 protein was initially purified from human erythrocytes and is a single polypeptide chain with an estimated molecular weight of 38.2 kDa (42kDa by SDS-gel electrophoresis) (Daddona and Kelley, 1977). It is coded for by the 32-Kb ADA gene on chromosome 20q13.11 which is composed of 12 exons (Petersen et al., 1987). Monomeric ADA1 consists of a polypeptide chain folded in α/β barrels that surround the active site, in which substrates are stabilised by hydrogen bonds, using Zn^{2+} as a cofactor (Fig. 5) (Wilson et al., 1991). ADA1 can also exist as a heterooligomeric dimer which has an estimated

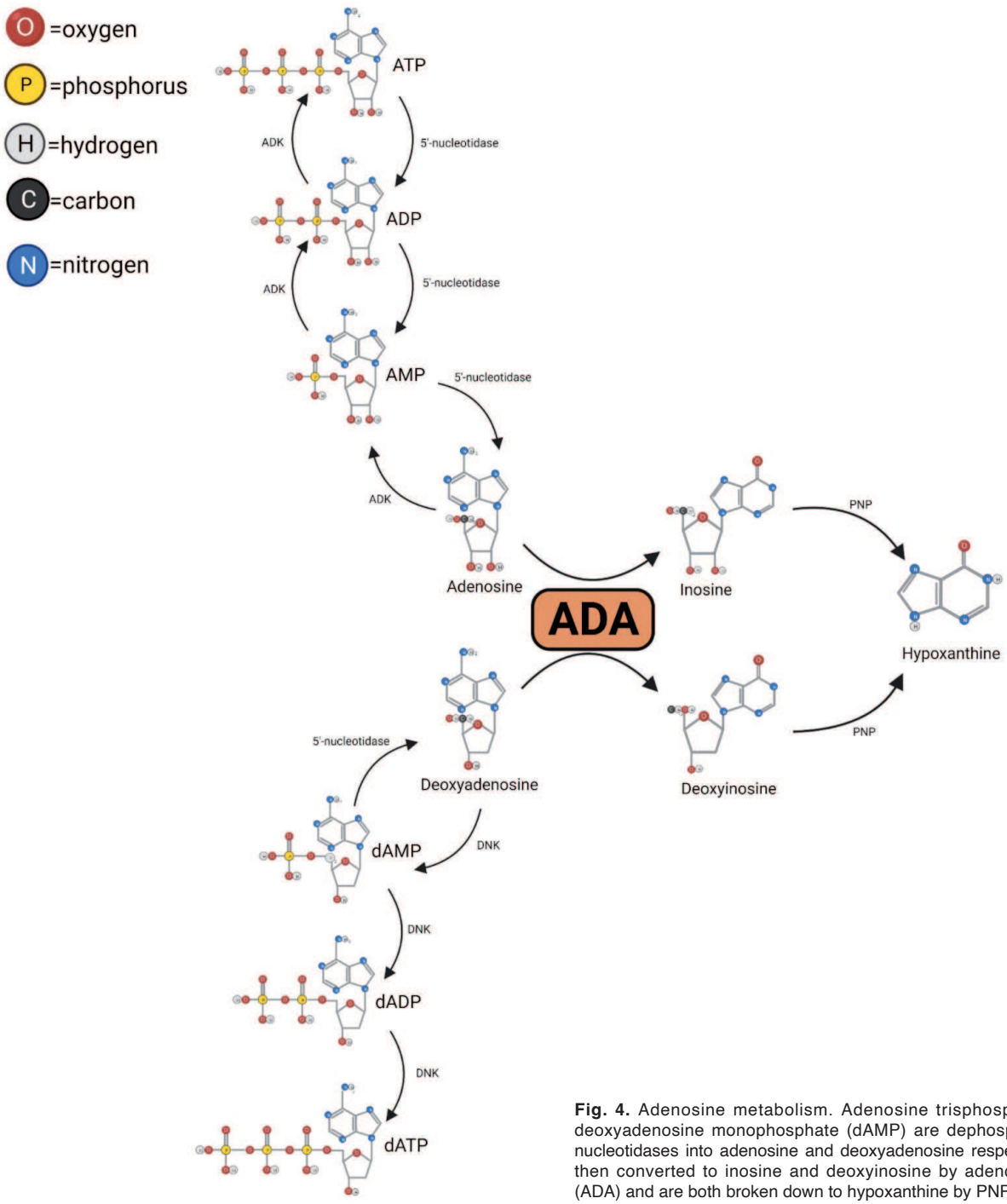

Fig. 4. Adenosine metabolism. Adenosine trisphosphate (ATP) and deoxyadenosine monophosphate (dAMP) are dephosphorylated by 5'-nucleotidases into adenosine and deoxyadenosine respectively. They are then converted to inosine and deoxyinosine by adenosine deaminase (ADA) and are both broken down to hypoxanthine by PNP.

molecular weight of 213kDa, and consists of two ADA subunits bound to dipeptidyl peptidase IV (DPP4/CD26) on the cell surface, facilitating the extracellular breakdown of adenosine (Fig. 3) (Kameoka et al., 1993; Weihofen et al., 2004). ADA2, also known as ADA related growth factor (ADGF) in insects and first identified in the spleen (Schrader et al. 1978), is mechanistically similar to ADA1, also catalysing the breakdown of adenosine and deoxyadenosine. However, it is coded for by the CECR1 (cat eye syndrome chromosome region, candidate 1) gene (Riazi et al., 2000), now referred to as the ADA2 gene (Ombrello et al., 2019) that spans 10 exons on chromosome 22q11.1. The ADA2 protein exists as a comparatively complex homodimer, with a unique α helical domain located in the N-terminal region. This mediates the dimerisation of its two identical subunits (Zavialov et al., 2010a), giving it an estimated molecular weight of 100kDa (Ratech et al., 1981). Despite low sequence homology, ADA2 is structurally similar to ADA1, forming an eight stranded parallel β-sheet surrounded by an α/β, TIM barrel (Zavialov et al., 2010a). The active site is functionally similar to ADA1 but has a markedly different hydrophobic binding pattern, differing in both the structure of ligand binding segments and in distribution of the hydrophobic sidechains (amongst several other structural divergences), which lead to the specificity between certain inhibitors of ADA1 and ADA2 (Zavialov et al., 2010a). The contrasting active site structure means ADA2 has a 100-fold lower Km for adenosine, and an optimum pH of 6.8 (Zavialov and Engström, 2005) which is acidic compared to ADA1's optimum pH of 7-7.4 (Van Der Weyden and Kelley, 1976). The isomers also differ in their distribution, ADA1 is ubiquitous in humans whereas ADA2 is active only in monocytes-macrophages, coexisting with ADA1 (Ungerer et al., 1992).

Conservation

ADA1 is an ancient enzyme, expressed by both prokaryotes and eukaryotes (Kathiresan et al., 2013). Recent phylogenetic analysis of over 240 genomes has indicated that whilst ADA1 is widespread, it may not be universal as it was not detected in plants, low fungi, some insect species, and some pathogenic eukaryotes (Skaldin et al., 2018). ADA2 was found in higher fungi and most animals, but with a non-uniform phylogenetic distribution, suggesting that ADA1 may compensate for the loss of ADA2 cell signalling function as ADA1 can be found extracellularly (Franco et al., 1997). Data also indicates that ADA2 is an ancient protein, originating in prokaryotes and may have been transferred between bacteria by horizontal gene transfer. Bioinformatic analysis suggested that bacterial ADA2 is a close homologue of eukaryotic ADA2 predicting homodimer formation, secretion into the extracellular space, and similar catalytic activities of both proteins (Dolezal et al., 2005).

As ADA1 is the most common and widely researched isoenzyme, further reference to 'ADA' will therefore refer to ADA1 and any mention of ADA2 will be specified.

Regulation

The regulation of ADA is complex, as it is facilitated by several factors. This is due to the ubiquitous nature of

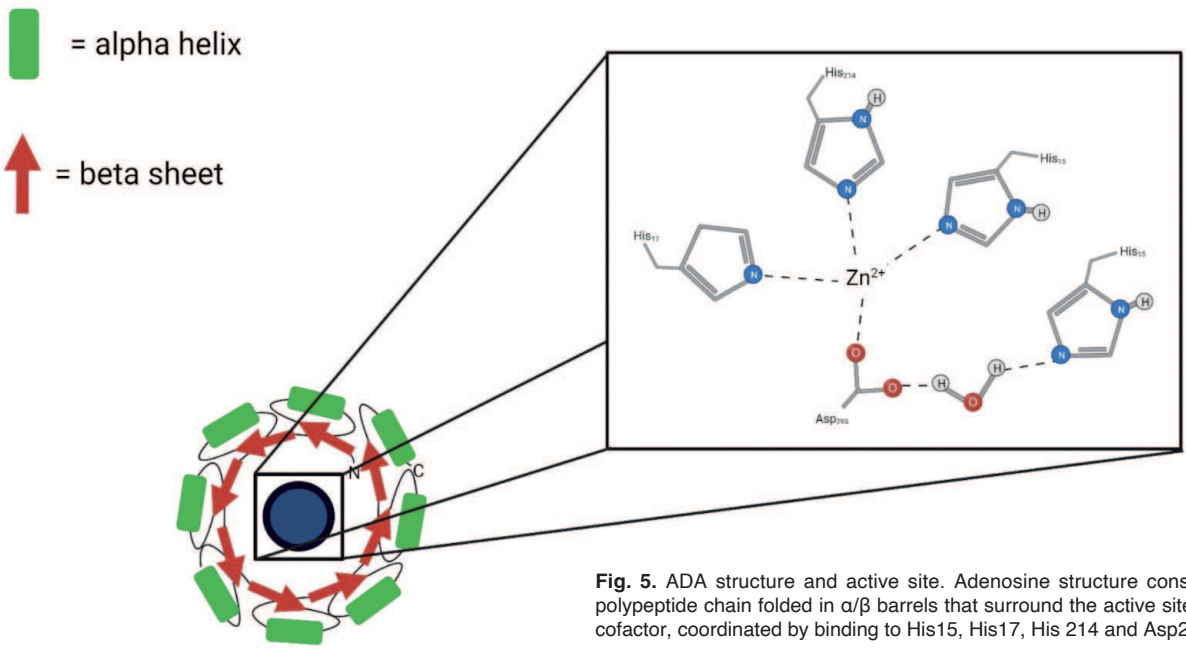

Fig. 5. ADA structure and active site. Adenosine structure consisting of a single polypeptide chain folded in α/β barrels that surround the active site, using Zn^{2+} as a cofactor, coordinated by binding to His15, His17, His 214 and Asp295.

the enzyme and its involvement in wide-ranging cellular processes.

Transcriptional regulation. The ADA gene has been proven to be a direct target of the transcription factors p63 and p73, homologs of the p53 tumour suppressor gene (Jost et al., 1997; Kaghad et al., 1997; Yang et al., 1998). The p63 and p73 genes code for several isoforms. Depending on the promoter p63 and p73 are transcribed from, they can have either an N-terminal transactivating domain (TA) (Kaghad et al., 1997; Yang et al., 1998) or a truncated N-terminal region that does not contain the TA-domain (Yang et al., 1998, 2000). p63 and p73 can also be spliced at the C-terminal region giving rise to several further isoforms (typified as α, β, γ etc.) (Murray-Zmijewski et al., 2006; Marshall et al., 2021). Reductions in ADA activity lead to an accumulation of deoxyadenosine, that in turn leads to a build-up of deoxyadenosine triphosphate (dATP), inhibiting ribonucleotide reductase and causing an imbalance in other dNTP molecules (Cohen et al., 1978). This causes disruption of both DNA repair and DNA synthesis which has been shown to lead to activation of p73α and β, increasing ADA mRNA activation by binding to intron 1 of the ADA gene through p53 responsive elements (Tullo et al., 2003). Inducible TAp73α expression in a human osteosarcoma SAOS-2 cell line led to an increase in ADA levels and concomitant purine metabolism changes including upregulation of adenosine and inosine (Tullo et al., 2003). Similarly, p53 responsive elements that interact with TAp63α and δNp63α have been reported in the ADA gene promoter region - overexpression of these isoforms confers transcriptional activation in MCF and 293T-Rex lines, whilst in epidermal keratinocytes, p63 knockdown correlated with a decrease in mRNA and protein levels of ADA (Sbisà et al., 2006). These data imply that modulating p73 and p63 levels may directly affect ADA activity, and the process may involve feedback mechanisms via the purine salvage pathway, as it is unclear how an increase in ADA levels causes adenosine levels to rise, as presented in Tullo et al. (2003).

ADA regulation has also been linked with the transcription factor Sp1 through binding of Sp1 to the ADA gene promoter (Xie et al., 1999). Sp1 regulates cell differentiation, immune signalling, DNA repair, apoptosis, and chromatin remodelling. Dusing and Wiginton (1994) demonstrated that the ADA promoter region contains six Sp1 binding sites. Knockout of these sites also showed that Sp1 was necessary but not sufficient by itself for high levels of ADA expression (Dusing and Wiginton, 1994). The role of p73 and Sp1 in the transcriptional regulation of ADA may have important implications for the neurodegenerative disorder ALS, which will be discussed later in this review.

Cell signalling regulation. Activity of ADA has been linked to the mitogen activated protein kinases (MAPK) pathway (Eguchi, 2020). The MAPK signalling pathway is activated in protein kinase cascades which consist of extracellular signal-regulated kinases (ERKs), Jun amino-terminal kinases (JNKs), and stress activated protein kinases (p38/SAPKs) (Boulton et al., 1990; Dérijard et al., 1994; Han et al., 1994). MAPK signalling is an important regulator of cell proliferation, differentiation, and death (for a review see Morrison, 2012). Eguchi et al., (2020) demonstrated that fibroblast growth factor 2 (FGF2), binding to FGF receptors, activated tyrosine kinase signalling, leading to downstream activation of ERK, JNK and p38. This induced an increase in ADA expression and activity (along with CD73/ecto-5'-nucleotidase which catalyses extracellular breakdown of AMP (Fig. 3)) in rat spinal cord astrocytes, whilst inhibition of both the FGF2 receptor and MAPKs downregulate expression (Eguchi, 2020). These data demonstrate that the FGF2/MAPK pathway is an important regulator of ADA amongst other purine metabolism enzymes.

Evidence has also shown that ADA expression levels can be regulated by 17Beta-oestradiol (E2), a member of the oestrogen family and a hormone that also modulates the expression of enzymes in the purine and pyrimidine biosynthesis pathways. Treating MCF-7 human breast cancer cells with E2 induces ADA mRNA activation, a finding that can be recapitulated with the use of tamoxifen - a breast cancer treatment drug (Xie et al., 1999). As with p73/Sp1, the role of oestradiol in the regulation of ADA levels may have important implications for ALS, which will be discussed later in this review.

Function

As previously stated, ADA functions within the purine salvage pathway, but it also plays a role in regulation of the immune response through its control of adenosine levels. Pharmacological estimates of basal extracellular adenosine concentrations typically lie between 25-250nM (Dunwiddie and Masino, 2001), but in instances of cell stress such as hypoxia or tissue damage, adenosine levels spike rapidly (Winn et al., 1981). This spike in adenosine leads to immunosuppression via activation of the A2A receptor, leading to an accumulation of intracellular cAMP and inhibition of the immune response (Henney and Lichtenstein, 1971), thus preventing edema and excessive inflammation (Sitkovsky and Ohta, 2005; Fredholm, 2007). Persistently high levels of adenosine can conversely lead to tissue damage (Van Linden and Eltzschig, 2007) and ADA tethered to DPP4 therefore functions to reduce potentially harmful extracellular adenosine levels and prevent chronic activation of adenosine receptors.

ADA also plays a key role in the differentiation and function of immune cells. Monocytes are white blood cells that can differentiate into macrophages and dendritic cells. During the early stages of monocyte

maturation and differentiation into macrophages, ADA activity is significantly increased (Fischer et al., 1976) with $ADA^{-/-}$ mice developing aberrant dendritic cells that have proangiogenic and proinflammatory properties (Novitskiy et al., 2008). ADA2 has also been shown to induce monocyte to macrophage differentiation (Zavialov et al., 2010b) suggesting both ADA and ADA2 play an important role in immune cell differentiation. ADA has also been shown to be required for macrophage activation by regulating superoxide generation, a process that is key for killing phagocytosed bacteria (Johnston Jr. et al., 1975), as inhibition of ADA in guinea pigs prevents superoxide generation (Yagawa and Okamura, 1981) and ADA activity correlates with total superoxide generation (Tritsch and Niswander, 1981).

In T-lymphocytes an accumulation of deoxyadenosine due to loss of ADA leads to increased levels of dATP, which concomitantly inhibits DNA synthesis, preventing T-lymphocyte differentiation (Carson et al., 1979). ADA also facilitates the immune response of T-lymphocytes by breaking down extracellular adenosine which prevents T-lymphocyte adenosine receptor activation, inhibiting immunosuppression (Dong et al., 1996). Moreover, ADA can activate the immune response by sending costimulatory signals to T-cells via DPP4 binding (Martín et al., 1995). ADA also 'bridges' between adenosine receptors on dendritic cells and DPP4 on T-cells (Pacheco et al., 2005; Moreno et al., 2018), which can induce T-lymphocyte proliferation and increase the production of proinflammatory cytokines (Pacheco et al., 2005). This process also leads to the increased generation of T-effector cells, T-memory cells and regulatory T-cells (Martinez-Navio et al., 2011). ADA is also key for B lymphocyte differentiation as $ADA^{-/-}$ mice develop B lymphocytes with proliferative, activational and structural defects and an increasing propensity to undergo apoptosis, likely caused by dATP and S-adenosylhomocysteine (SAH) accumulation as observed in T-lymphocytes (Aldrich et al., 2003). This demonstrates the vital function of ADA in not only immune cell generation but also in overall function and makes ADA an important choreographer of the immune response in the body.

ADA in the CNS

ADA can also act as a neuromodulator via the regulation of adenosine. Adenosine modulates activity in the brain via the G-protein-coupled receptors, A1, A2A, A2B and A3 with the A1 and A3 receptors coupling Gi/o receptors and A2A and A2B receptors coupling Gs receptors (Dunwiddie and Masino, 2001). This coupling means activation of A1 and A3 receptors inhibits adenylyl cyclase activation, preventing the conversion of ATP to cAMP; conversely activation of A2A and A2B receptors stimulates adenylyl cyclase activation, promoting cAMP production. The A1 receptor has the highest affinity for adenosine and is widely expressed in tissues of the brain (Dixon et al., 1996); its activation is coupled with the inhibition of Ca^{2+} influx (Dolphin et al., 1986) and the activation of K^{+} influx (Trussell and Jackson, 1985). This mechanism prevents the release of neurotransmitters such as dopamine, glutamate, and acetylcholine amongst others, effectively reducing excitability (Dunwiddie and Masino, 2001). ADA is also required for the coupling of the A1 receptor to heterooligomeric G protein receptors (Saura et al., 1996). A2A receptor expression in the brain is limited to the striatum, nucleus accumbens and olfactory tube (Dixon et al., 1996) and is also coupled with Ca^{2+} inhibition. The A2B and A3 receptors are also widely expressed in the brain but at very low levels (undetectable by in situ hybridisation in the rat brain (Dixon et al., 1996)) and have very low affinities for adenosine in comparison to the A1 and A2A, receptors and are thus less well characterised. However, activation of A2B in the CNS has recently been shown to improve intestinal barrier function via the vagus nerve (Ishioh et al., 2021) and protect against ischemic damage (Dettori et al., 2021); and A3 receptor activation can induce a PKC-dependent inhibition of group 3 metabotropic glutamate receptor (mGluR) function at the Schaffer collateral-CA1 synapse inhibiting neurotransmission (Macek et al., 1998). The physiological roles of adenosine in the brain include the regulation of the sleep-wake cycle (Huang et al., 2014), coupling cerebral blood flow with energy demands (Winn et al., 1981); modulating synaptic plasticity (Sebastião et al., 2001), the prevention/repair of ischemic damage (Rudolphi et al., 1992), motor function (El Yacoubi et al., 2000), astrocyte function (Florian et al., 2011), aging (Castillo et al., 2009; Costenla et al., 2011) and feeding (Lee et al., 2005).

Any disturbance therefore in the regulation of adenosine can have catastrophic effects on homeostasis in the body and particularly in the brain, hence the prominent role ADA aberration plays in various disease pathologies.

ADA in disease

Mutations and splicing

Mechanisms leading to ADA deficiency are caused by alterations at the level of transcription, translation, or alterations in the protein itself. A reduction in the levels (or complete loss) of ADA protein can arise through mutations that repress transcription of the ADA gene, or decrease the stability of the encoded mRNA or protein. Whilst mutations that reduce substrate or cofactor (Zn2+) binding in the active site, or change key catalytic residues, have also been reported, that give rise to ADA with reduced enzymatic activity. Alternatively, in the absence of changes to the nucleotide sequence, altered epigenetic regulation of the ADA gene may be responsible for increases or decreases in ADA levels.

A high number of ADA mutations lead to severe combined immunodeficiency (SCID) (Atasoy et al., 1993; Santisteban et al., 1993; Hershfield, 2003; Kalman et al., 2004) which will be discussed further. These mutations arise from premature stop codons, DNA deletions or insertions, amino acid substitutions, RNA splicing defects and post-translational modification defects. In terms of splicing, it has been historically hypothesised that, in spite of mutations being present in people, low levels of "normal" pre-mRNA splicing may still occur. Moreover, the level of splicing efficiency may be linked to ADA activity levels and therefore clinical severity even between siblings (Santisteban et al., 1993; Arredondo-Vega et al., 1994). A mutation in the last acceptor splice site in the ADA gene has been shown to lead to aberrant splicing, which altered the structure of the ADA protein, adding a short tail residue section leading to protein instability, loss of ADA activity and disease. Interestingly, an 11 base pair deletion adjacent to the g.31701T>A mutation in one sibling pair suppressed aberrant splicing, increasing ADA activity and protein stability (Arredondo-Vega et al., 2002). Enhanced splicing in ADA has also been observed. Several genes contain purine-rich exonic regions which interact with splicing factors and cis-acting intronic elements defining exons (Lavigueur et al., 1993; Mayeda et al., 1993; Tian and Maniatis, 1993, 1994; Watakabe et al., 1993; Staknis and Reed, 1994; Tanaka et al., 1994). It has been reported that an ADA R142X mutation located within a purine-rich region of exon 5, caused exon skipping, possibly by splicing enhancer disruption (Santisteban et al., 1995). G to A transition at nucleotide 22 of exon 1 of the ADA gene gives rise to an Asp to Asn amino acid substitution in position 8 of the mature protein. Although rare (allelic frequency of 0.03–0.11 in Caucasian populations), this reduces ADA activity by 35% compared to the Asp allozyme (Hirschhorn et al., 1994).

ADA and links to diabetes, haemolytic anaemia, pulmonary fibrosis and cardiovascular disease

Type two diabetes (T2DM), which is caused by insulin resistance (Roglic, 2016) has been linked to higher serum ADA activity (Hoshino et al., 1994; Kurtul et al., 2004; Lee et al., 2011; Niraula et al., 2018) as ADA expression negatively correlates with insulin levels (Rutkiewicz and Górski 1990). Neurologically, T2DM has been linked to an increased likelihood of developing Alzheimer's disease (Sims-Robinson et al., 2010), and conversely with a decreased likelihood of developing ALS, which will be discussed further in section 2.7.

Evidence also suggests that higher erythrocyte ADA activity may lead to decreased adenine pools in people with mild chronic haemolytic anaemia, resulting in premature red blood cell death (Valentine et al., 1977; Chottiner et al., 1987) which may be related to aberrant ADA mRNA translation in red blood cells (Chottiner et al., 1987). Neurological involvement is observed in 20-50% of patients in haemolytic anaemia and has been linked to increased mortality (Sheth et al., 1986).

ADA is also involved in the progression of pulmonary fibrosis. Chunn et al. (2005) showed that $ADA^{-/-}$ mice had extensive pulmonary inflammation and increased lung adenosine levels which could be reversed via enzyme replacement therapy (ERT) preventing the development of pulmonary fibrosis (Chunn et al., 2005).

An increase in ADA activity has also been linked with several cardiovascular disorders including atherosclerosis, acute myocardial and ischemic reperfusion injury, thrombosis, and hypertension (along with T2DM that can cause cardiovascular disorders). Because of this, ADA inhibitors such as erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) have been proposed for use in cardioprotective therapies, but due to the poor pharmacokinetics and toxicity induced by ADA inhibition, this has so far proved unsuccessful (for a review see Kutryb-Zajac et al., 2020).

ADA-deficient severe combined immunodeficiency

ADA deficiency is the second most common cause of SCID, accounting for 15% of all cases (for a review see Hershfield, 2017). ADA-deficient SCID is an inherited autosomal recessive disease caused by complete or partial loss of ADA activity (Giblett et al., 1972). Over 70 causative mutations in the ADA gene have been identified that give varying levels of ADA activity in the host (Arredondo-Vega et al., 1998; Flinn and Gennery, 2018) and lead to the three forms of the disease: Early onset SCID, which represents most cases, presents before the child reaches 12 months and leads to the most severe symptoms (Giblett et al., 1972); delayed/late-onset SCID, which develops between years 1-10, in which symptoms are less acute (Geffner et al., 1986); and the benign condition, partial ADA deficiency (Jenkins et al., 1976). ADA-deficient SCID has two major pathogenic mechanisms. Firstly, via an accumulation of dATP, inhibiting T-lymphocyte proliferation (Carson et al., 1979). Secondly, adenosine can combine with homocysteine to form SAH (Fig. 3). Accumulation of adenosine causes a subsequent accumulation of SAH that inhibits SAM generation and therefore SAM-mediated DNA methylation, a process which is required for normal thymocyte differentiation (Benveniste et al., 1995). SCID results in the almost total depletion of the body's immune response and can have devastating effects on the host (Giblett et al., 1972). However, all forms of SCID can be treated with allogeneic hematopoietic stem cell transplantation (HSCT) and bone marrow transplant (BMT), ERT using ADA conjugated to polyethylene-glycol (PEG-ADA) (Hershfield, 2017), and through lentivirus mediated autologous hematopoietic stem cell gene therapy (HSC-GT) (Aiuti et al., 2009).

Along with the devastating immunological implications of SCID, patients can also experience severe neurological manifestations, a phenomenon that

is particularly relevant for ADA-deficient SCID patients and which can persist even after treatment. ADA-deficient SCID patients have lower IQ scores than patients with other forms of SCID and the general population (Rogers et al., 2001; Titman et al., 2008; Sauer et al., 2017); exhibit behavioural abnormalities including hyperactivity disorder like symptoms, aggressive behaviour and social problems, not reported in other forms of SCID (Rogers et al., 2001; Hönig et al., 2007; Scott et al., 2017); motor dysfunction with symptoms including hypotonia and nystagmus (Hirschhorn et al., 1980; Hönig et al., 2007; Nofech-Mozes et al., 2007) and also auditory dysfunction (Tanaka et al., 1996). The neurological defects observed in SCID are overshadowed by the profound immunological changes that occur in early childhood meaning little research has been conducted into the changes that occur neurologically in sufferers. Therefore, the exact mechanisms of the neurological manifestations of ADA-deficient SCID are unknown. It has been noted that patients' IQ scores were inversely correlated with dATP levels at the time of diagnosis (Rogers et al., 2001), implying that the neurological manifestations of ADA-deficient SCID are caused by or at least correlated with levels of toxic metabolite accumulation caused by the loss of ADA activity. Sauer et al. (2017) however, showed there was no apparent correlation between dATP levels, instead linking the observed neurological dysfunction with A2A receptor activation as caffeine, which interacts with the A2A receptor, abolished an anxiogenic phenotype in $ADA^{-/-}$ mice (Sauer et al., 2017). MRI and tomographic scans also reveal volume loss of the basal ganglia and thalamus, possibly linked to atypical adenosine receptor activation in patients (Nofech-Mozes et al., 2007).

The persistence of the neurological manifestations of SCID post-treatment may in part be because ERT cannot restore ADA levels in the brain as PEGylated ADA cannot cross the blood brain barrier (Sauer et al., 2017), whilst BMT is known to induce neurotoxicity when performed at an early age, which appears to only augment the neurological impairment of ADA-deficient SCID patients (Rogers et al., 2001; Titman et al., 2008). Even after HSC-GT treatment, the majority of participants continue to report neurological impairment (Aiuti et al., 2009; Cicalese et al., 2016). Due to the influence ADA exerts in control of the CNS, specifically in its control of adenosine and adenosine's interaction with its receptors in the brain (Nagy et al., 1984), it is no surprise that ADA-deficient SCID patients exhibit these neurological defects, as any disturbance in the delicate balance of this system is likely to have significant, lasting effects.

ADA2 deficiency

Deficiency of ADA2 (DADA2) is an autosomal recessive disorder, caused by loss-of-function mutations in the ADA2 gene leading to reduction in enzyme activity (Navon Elkan et al., 2014; Zhou et al., 2014). The disease presents in early childhood and symptoms include autoinflammatory, vasculopathic, hematologic and immune system dysfunction (Ombrello et al. 2019). Zhou et al. (2014) showed that monocytes from patients could differentiate in to proinflammatory M1 macrophages but not anti-inflammatory M2 macrophages which presumably stems from the role ADA2 carries out in monocyte to macrophage differentiation (Zavialov et al., 2010b; Zhou et al., 2014). It has also been shown that neutrophil extracellular trap formation, induced by adenosine signalling, may contribute to the observed vasculopathy in DADA2 (Carmona-Rivera et al., 2019).

DADA2, like ADA-deficient SCID, also has a range of neurological manifestations. Ischemic strokes are a common feature (Zhou et al., 2014) with imaging of the brain showing lacunar lesions in the brain stem (Bulut et al., 2019), the effects of which can accumulate over time to induce more severe neurological symptoms such as dysarthria, ataxia, palsy, and cognitive impairment (Springer et al., 2018). Other neurological manifestations have included intracerebral haemorrhaging (Belot et al., 2014; Garg et al., 2014; Navon Elkan et al., 2014), central and peripheral neuropathy (Lee et al., 2018) and aneurysm (Navon Elkan et al., 2014). Neuroimaging has also more recently demonstrated that patients develop cerebral microbleeds and inflammatory perivascular tissue in the basal and prepontine cisterns (Geraldo et al., 2021).

Autism

Historic evidence has correlated lower ADA levels and autism (Stubbs et al., 1982). Moreover, an increase in the frequency of the previously discussed Asp8Asn polymorphism which reduces ADA activity was observed in Italian children diagnosed with autism suggesting that this genotype-dependent reduction in ADA activity may be a risk factor for the development of the disease (Bottini et al., 2001). These findings may be population dependent as a similar study on a North African cohort did not produce such a clear link between the polymorphism and autism (Hettinger et al., 2008), whereas a study in Saudi Arabia found decreased ADA levels in the plasma of autistic boys (Abu Shmais et al., 2012). More recently a zebrafish model of autism linked a dysfunction in ADA with disease pathogenic mechanisms including altered intracellular and extracellular purine metabolism (Zimmermann et al., 2016).

Parkinson's Disease

Evidence has shown that ADA levels are also dysregulated in Parkinson's disease (PD). In a paper published in 1995, serum isolated from idiopathic PD patients was shown to have significantly higher total ADA and ADA2 activity levels compared to controls

(Chiba et al., 1995). Moreover, levels correlated with activated T-lymphocyte populations, suggesting peripheral T-lymphocyte activation was the cause. Similar results were observed more recently in a metabolomics study performed in mice treated with lipopolysaccharide plus 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which evokes a PD like response in rodent models (Huang et al., 2019). Widespread metabolic alterations were observed including in purine metabolism where adenosine levels were decreased, and inosine levels increased. ADA inhibition by deoxycoformycin and/or A2A antagonism with KW6002 reduced dopamine loss and dopaminergic cell death and improved motor function. These data demonstrate that targeting dysregulated purine metabolism by ADA regulation is a potential therapeutic approach in PD by reducing inflammatory pathways. These mechanisms may be similar to the action of caffeine which is known to be neuroprotective via A2A (for a recent review see Ren and Chen, 2020).

Amyotrophic lateral sclerosis

ALS is a neurodegenerative disease characterised by the death of motor neurons (MNs) in the brainstem and spinal cord (for a recent review see van Es et al., 2017). The disease is incurable and only two therapeutics currently exist for its treatment, Riluzole and Edaravone, both of which have modest effects on disease progression (Bensimon et al., 1994; Abe et al., 2017). The lack of efficacious therapies for ALS stems from its complex aetiology. The disease can be divided into two classifications, familial ALS (fALS) which accounts for 5-10% of all cases and sporadic ALS (sALS) that accounts for 90-95% of all cases (Mulder et al., 1986). Genetically over 50 genes are associated with the development of ALS (Mejzini et al., 2019), the most common of these is a hexanucleotide repeat expansion of GGGGCC in the chromosome 9 open reading frame 72 (C9orf72) gene, that codes for the C9orf72 protein and accounts for 33% of fALS and 5% of sALS cases (Majounie et al., 2012). C9orf72 has been hypothesised to lead to ALS via three main mechanisms, either through toxic loss of function in the C9orf72 protein (for a recent review see Xu et al., 2021) or toxic gain of function from RNA foci transcribed from the repeat expansion and in non-AUG RAN translated dipeptide repeat (DPR) proteins (Mori et al., 2013), that cause RNA processing errors and nucleolar structure damage amongst a host of other potential pathogenic mechanisms (Balendra and Isaacs, 2018).

Dysregulated adenosine signalling influences ALS pathology and the role of adenosinergic and purinergic receptors has been widely studied in relation to ALS (for reviews see Cieślak et al., 2018; Sebastião et al., 2018). Recent investigations from our laboratory have indicated that loss of ADA may also contribute to the pathology of the disease (Allen et al., 2019). Astrocytes are neuronal support cells that have been shown to have a significant effect on disease pathology in ALS due to the reliance of MNs on astrocytes for energy amongst other functions (McGeer et al., 1988). In our laboratory we metabolically profiled ALS patient derived iAstrocytes showing that ALS iAstrocytes have reduced adenosine metabolism compared to controls (Allen et al., 2019). Further investigation demonstrated that ADA protein expression was significantly lower in both C9orf72 and sALS iAstrocytes and ALS iAstrocytes were significantly more susceptible to adenosine-mediated toxicity. The data here suggests an important role in ALS for ADA and cements ADA as an increasingly important enzyme in the brain.

Future work

ADA dysregulation in ALS

A key question we are currently investigating in our laboratory is, what causes loss of ADA in ALS? Furthermore, are the mechanisms similar or distinct in familial and sporadic disease? Compelling historic and recently published studies have suggested a link between disease mechanisms and ADA, which we will discuss in this section and have been summarised in Fig. 6.

What causes loss of ADA in ALS?

The finding that ALS astrocytes show a reduction in ADA levels may be related to loss of transcriptional regulation. Russell et al. (2021) utilising exome sequencing on a cohort of 87 sALS patients against 324 control cases identified 5 missense single nucleotide variants (SNVs) that were potentially ALS causing pathogenic variants in the transcription factor p73. The finding was subsequently confirmed in a cohort of 53 and 2,800 further ALS patients on which exome sequencing identified 19 further rare, nonsynonymous variants. In total, 22 missense SNVs and 2 in-frame indels were found in the three cohorts (Russell et al., 2021). Four variants were then chosen to be modelled which were cloned into δN-p73α and expressed in C212 myoblast lines. Two of the four mutant lines were demonstrated to inhibit differentiation in the myoblasts. p73 knockout in a zebrafish model induced apoptosis which led to a reduction in spinal MN levels and in spinal motor neuronal axon branching (Russell et al., 2021). $p73^{-/-}$ mice show signs of severe neurological defects along with immune and inflammatory dysfunction (Yang et al., 2000; Wilhelm et al., 2010) and p73 was proven to be important for neuronal survival (Tissir et al., 2009) likely because of its anti-apoptotic effects which counteract the effect of p53 (Pozniak et al., 2000). In addition, $p73^{+/-}$ Alzheimer's models have established that p73 may be required to protect against neurodegeneration (Wetzel et al. 2008; Cancino et al., 2013) and induction of p73 may be neuroprotective (Shekhar and Dey, 2019). Though some studies have disputed its importance, specifically in the pathogenesis

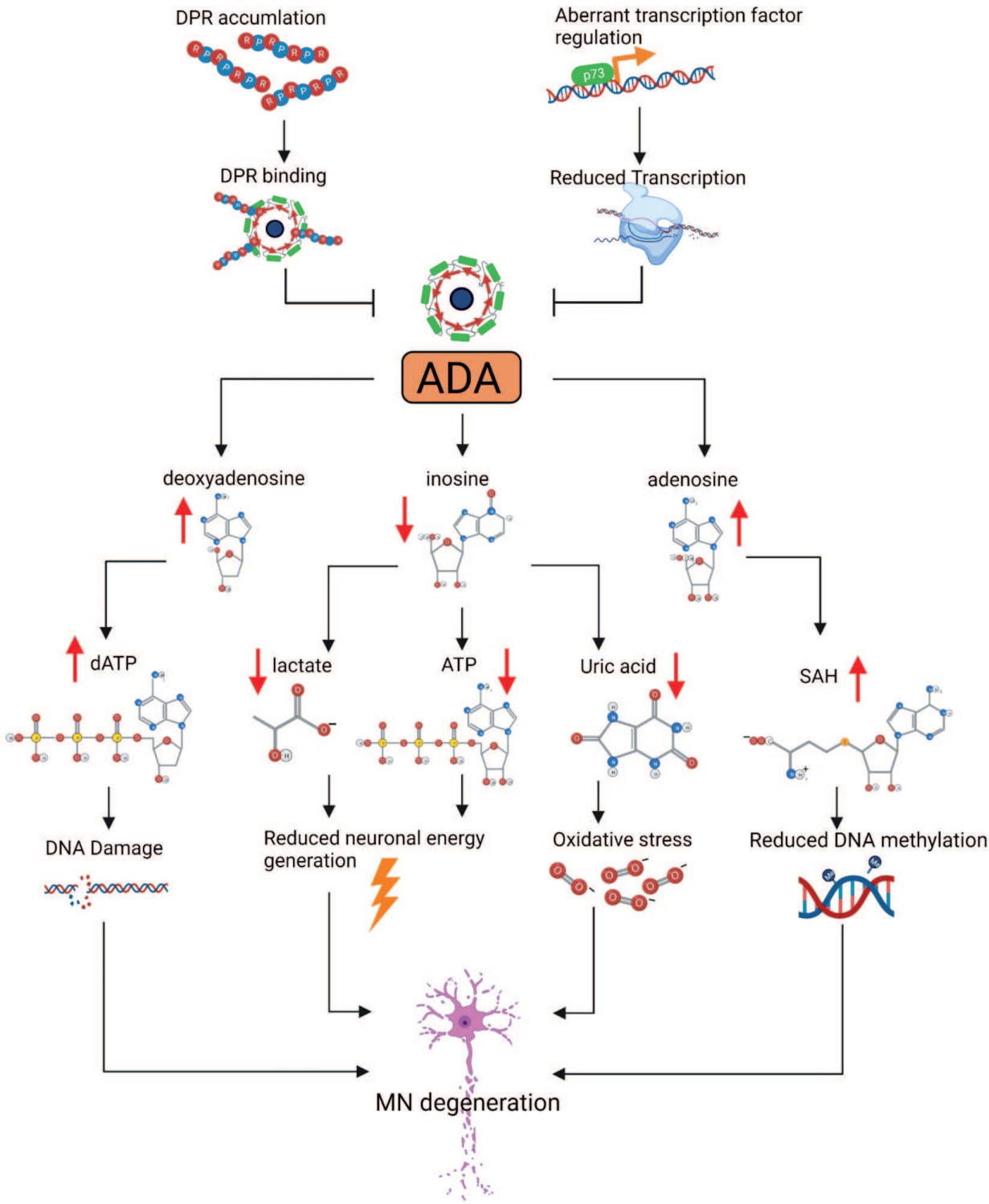

Fig. 6. Possible mechanisms of ADA mediated motor neuron degeneration. Loss of ADA in ALS may lead to MN degeneration via several mechanisms. p73 dysregulation would lead to lower ADA gene transcription and DPR accumulation could lead to poly-PR ADA binding, both reducing ADA protein function. This would lead to a decrease in inosine output and an accumulation of adenosine and deoxyadenosine. Loss of inosine would result in a reduction in both inosine-mediated lactate and ATP output, and therefore energy generation in MNs which has been linked to ALS pathogenesis previously. It would also result in a reduction in uric acid levels, a potent antioxidant, which would contribute to the oxidative stress that has also been linked to ALS pathogenesis. Accumulations of deoxyadenosine and adenosine may also cause incorrect immune cell function and affect DNA synthesis and repair, and methylation in ALS astrocytes, another potential pathogenic mechanism behind MN degeneration.

of Alzheimer's (Vardarajan et al., 2013), these data indicate p73 may be an important factor in the pathogenesis of ALS and neurodegeneration in general (Fig. 6). As ADA loss has also been linked to ALS pathology (Allen et al., 2019) and p73 is a known regulator of ADA (Tullo et al., 2003), it could be inferred that the link between ALS and ADA is controlled by defective p73 regulation leading to loss of ADA. Future work in this area is ongoing in our laboratory to attempt to identify the relationship between ALS pathogenesis and ADA regulation via p73.

Another possible pathway for dysregulation of ADA in ALS would be via the transcription factor Sp1. There is little information available on Sp1 in relation to ALS pathology, however a recent paper using combined transcriptomic analysis identified Sp1 as a possible driver of MN degeneration in ALS and a link between differentially expressed genes in the blood and brain tissue of ALS patients (Rahman et al., 2019).

It is difficult to confirm the link between ADA and ALS as minimal literature exists for the behaviour of ADA in ALS patients and models, there is however an abundance of evidence for dysfunction in ADA acting on RNA (ADAR). ADAR is an analogue of ADA and an enzyme that partakes in RNA editing via post-transcriptional modification, converting adenosine bases to inosine (Melcher et al., 1996). In ALS, glutamate excitotoxicity can be caused by a genetic variant in the AMPA receptor; it has been hypothesised that incorrect adenosine to inosine conversion at the pore-lining domain GluA2 of AMPA may underlie this toxicity (Aizawa et al., 2010) caused by significant disturbances in both ADAR2 expression (Hideyama et al., 2012) and localisation (Moore et al., 2019). A recent paper has indicated that in C9orf72 ALS this dysfunction is likely caused by proline-arginine poly-DPRs (poly-PR) binding to ADARs that inhibit the RNA editing ability of both ADAR1 and 2 in in vitro models (Suzuki and Matsuoka, 2021) - though what this means for ALS that stems from an alternative genetic origin is unclear and ADAR dysfunction might be driven by other mechanisms in other forms of the disease. Regardless, this suggests a precedent for incorrect ADA function in ALS and poly-PR binding could be a mechanism for ADA loss in C9orf72 ALS cells (Fig. 6).

Possible involvement of toxic metabolite accumulation in ALS?

A loss of ADA expression and activity in ALS would also suggest an accumulation of its substrates. The effect of this in ADA deficient patients could be an accumulation of dATP, leading to disrupted DNA repair and synthesis (Cohen et al., 1978) and accumulation of SAH, leading to reduced DNA methylation (Benveniste et al., 1995). DNA damage and its effects are well characterised in ALS (for a recent review see Kok et al., 2021). Elevated DNA damage has long been associated with sALS patients (Fitzmaurice et al., 1996; Ferrante et al., 1997; Bogdanov et al., 2000; Ihara et al., 2005; Mitsumoto et al., 2008; Murata et al., 2008; Blasco et al., 2017; Kim et al., 2020). Several ALS causing mutations have also been associated with DNA damage, inefficient DNA repair and a dysfunctional DNA damage response (DDR) (Fitzmaurice et al., 1996). Increased protein expression and staining for phosphorylated H2AX (γH2AX), a marker of DNA double strand breaks (Rogakou et al., 1999), has been observed in C9orf72 post-mortem spinal cord tissue, and C9orf72 neuronal and DPR cell models, possibly caused by oxidative stress or R-loop formation (Lopez-Gonzalez et al., 2016; Farg et al., 2017; Walker et al., 2017; Choi et al., 2019; Andrade et al., 2020; Nihei et al., 2020). Whilst DPRs have also been shown to inhibit DNA repair by inducing chromatin compaction or interfering with non-homologous end-joining, single-strand annealing, DNA repair via NPM1 and p53 function, that can mediate the DNA repair response (Farg et al., 2017; Walker et al., 2017; Andrade et al., 2020; Maor-Nof et al., 2021).

A mutation in the super oxide dismutase gene (SOD1), an ALS-causing gene (Rosen et al., 1993) has also been associated with elevated γH2AX and OpG, a measure of oxidative DNA damage in murine models (Kasai and Nishimura, 1984; Warita et al., 2001; Aguirre et al., 2009; Fang et al., 2010; Li et al., 2019). Which may be caused by both a loss and toxic gain of function (Sau et al., 2007; Barbosa et al., 2010; Brasil et al., 2018; Wang et al., 2019; Zhang et al., 2019). SOD1 mutations may impair the DDR by inducing the mislocalisation of several components of DDR (Li et al., 2019).

Mutations in proteins that are directly involved in DDR and DNA repair can also cause ALS and induce DNA damage. Fused in Sarcoma (FUS) regulates DNA repair and DDR in neurons (Wang et al., 2019). Post-mortem tissue from patients showed higher levels of DNA damage (Naumann et al., 2018; Wang et al., 2019), which may be caused by mislocalised FUS (Higelin et al., 2016) leading to impaired DDR and DNA repair. Elevated DNA damage has also been associated with mutations in TARDBP that codes for transactive response DNA binding protein (TDP43) (Guerrero et al., 2019; Konopka et al., 2020) that could also interfere with the DDR by associating with several proteins involved in DDR (Freibaum et al., 2010; Mitra et al., 2019). Never-in-mitosis A related protein kinase 1 (NEK1) (Kenna et al., 2016) is involved in the DDR (Polci et al., 2004) and leads to higher levels of DNA damage in NEK1-ALS derived MNs (Higelin et al., 2018). The above data indicate a clear association between DNA damage, insufficient DDR/DNA repair and ALS pathology. Loss of ADA, inducing dATP accumulation in ALS could represent a further link between insufficient DNA repair and ALS, possibly being a cause of or a contributor to DNA repair malfunctions in ALS.

Evidence correlating aberrant DNA methylation and ALS is less comprehensive and somewhat conflicting.

Several studies link ALS with hypermethylation (Xi et al., 2013; Tremolizzo et al., 2014; Coppedè et al., 2017; Hamzeiy et al., 2018) whilst other studies suggest no difference in methylation signatures in ALS patients (Oates and Pamphlett, 2006; Garton et al., 2017), and several demonstrate that there is both hyper- and hypomethylation in ALS patients (Morahan et al., 2009; Figueroa-Romero et al., 2012; Appleby-Mallinder et al., 2021). Other studies have also linked ALS with hypomethylation (Wong et al., 2013; Stoccoro et al., 2018, 2020). Wong et al. (2013) demonstrated that mitochondrial Dnmt3a, a DNA methyltransferase enzyme responsible for de novo methylation, had significantly lower expression levels in the skeletal muscle and spinal cord of SOD1 mouse models in presymptomatic and early disease stages (Wong et al., 2013). Significantly lower levels of D-loop methylation have also been observed in SOD1 and sALS patients (but not C9orf72 patients) which inversely correlate with mitochondrial DNA copy number (Stoccoro et al., 2018, 2020). Mice that lack Dnmt3a in the nervous system also go on to develop an ALS-like phenotype (Nguyen et al., 2007). These data suggest a role for hypomethylation in ALS but there are clearly wide aberrations in global DNA methylation that occur in ALS that include both hyper- and hypomethylation. It is therefore possible that there is a link between ADA-loss induced inhibition of SAM-mediated DNA methylation and ALS, though this may only be the case in certain cohorts, as hypomethylation is only observed in SOD1 models and SOD1 and sALS patients.

Is immune cell dysfunction in ALS associated with ADA?

Dysfunctional immune cell regulation is a common observance in ALS. Circulating monocytes from both sALS and fALS patients have been shown to demonstrate aberrant subtype regulation, incorrect adhesion, and dysregulated phagocytic activity (Zondler et al., 2016). Moreover, levels of monocytes with a proinflammatory phenotype have been shown to correlate with faster disease progression (Zhao et al., 2017). A reduced number of circulating dendritic cells were observed in sALS patients but were also predicted to be proinflammatory (Rusconi et al., 2017), whilst dendritic cell levels were significantly increased in spinal cord tissue taken from both fALS and sALS patients, which also positively correlated with disease progression (Henkel et al., 2004). Reduced expression of T-lymphocytes has been observed in sALS patients (Mantovani et al., 2009), regulatory T-cells taken from patients were shown to be dysfunctional (Beers et al., 2017) and T-lymphocyte levels may negatively correlate with accelerated disease progression (Henkel et al., 2013). The importance of ADA in immune cell differentiation and function has been discussed in section 1.2.3. and in general, the role that ADA plays in immune cell regulation is well established (for a review see Antonioli et al., 2012), as evidenced by severe disruption of immune cell function in ADA-deficient SCID (Hershfield, 2017). Immunodeficiency has recently been linked to ALS (Béland et al., 2020) and the immune cell dysfunction outlined here could therefore be linked to ADA dysregulation. However, it may be that the level of ADA loss in ALS is cell dependent. Data from our laboratory suggested that ADA loss was more severe in astrocytes compared to neurons and more severe in neurons compared to fibroblasts (Allen et al., 2019). Therefore, it remains to be seen whether loss of ADA is observed both in immune cells and for example in microglia and whether this negatively influences ALS disease pathology. More work is required in this area.

Can ADA manipulation be protective in ALS?

Recent work from our laboratory demonstrated a loss of ADA in ALS astrocytes that lead to an increased susceptibility to adenosine mediated toxicity (Allen et al., 2019). We also demonstrated that inosine supplementation was beneficial bioenergetically for the iAstrocytes and inosine supplementation was able to ameliorate iAstrocyte-mediated toxicity to MNs in co-culture and that ADA levels negatively correlated with adenosine mediated toxicity in ALS iAstrocytes and positively correlated with MN survival in the presence of inosine (Allen et al., 2019). This suggests that higher ADA activity in addition to reducing toxic adenosine levels would produce more inosine and may be protective in ALS. With these data in mind, we recently showed that inosine metabolism positively correlates with disease duration in ALS fibroblasts (Gerou et al., 2021). Elevated inosine production could be beneficial in two major ways. Firstly, lactate produced by astrocytes is used by MNs as a source of energy (Pellerin and Magistretti, 1994) and dysfunctions in lactate metabolism have been linked to ALS previously (Ferraiuolo et al., 2011, 2016). This is important as inosine can be converted to ribose-1-phosphate that contributes to glycolysis via the pentose phosphate pathway, producing ATP, NADH and eventually lactate (Jurkowitz et al., 1998; Balestri et al., 2007). This could therefore form part of the mechanism by which MN survival is enhanced when iAstrocytes are supplemented with inosine in MN/astrocyte co-cultures and enhanced inosine metabolism is protective in ALS (Allen et al., 2019; Gerou et al., 2021). Secondly, inosine can be converted to uric acid via conversion to hypoxanthine and subsequently xanthine (Fang et al., 2013). Uric acid is hypothesized to be a potent antioxidant (Ames et al., 1981) which may be neuroprotective (Chen et al., 2012, 2013) and in ALS patients uric acid levels correlate with disease progression (Keizman et al., 2009; Paganoni et al., 2012; Oh et al., 2015). This suggests uric acid has an important role in ALS pathology, though increased uric acid is unlikely to be the mechanism by which inosine supplementation can reduce ALS iAstrocyte-mediated

toxicity to MNs, as uric acid level did not correlate with increased MN survival in co-cultures (Allen et al., 2019). These data therefore suggest that increased ADA levels would be beneficial for ALS patients, either from increased toxic metabolite breakdown or through increasing inosine levels and therefore uric acid and lactate output, or both (Fig. 6).

In possible confirmation of the therapeutic benefit of elevated ADA levels in ALS patients, recent evidence suggests a protective role for oestradiol in ALS in pre-menopausal women (Klemann et al., 2018). Moreover, women with higher lifetime endogenous oestrogen exposure were associated with a longer survival in ALS (de Jong et al., 2012) and treatment with 17beta-oestradiol was shown to be protective in a SOD1 mouse model (Heitzer et al., 2017). 17beta-oestradiol has also been shown to protect against demyelination and axonal injury in MS mouse models (Aryanpour et al., 2021) and may be protective in other neurodegenerative diseases (Garcia-Segura et al., 2001). As discussed in section 1.2.2.2, oestradiol has been shown to increase ADA levels (Xie et al., 2001) insinuating a purine metabolism related mechanism for oestrogen's neuroprotection in ALS. Further investigation could focus on the potential role that oestradiol plays in regulation of ADA which may facilitate its effect on ALS.

Several studies have also associated T2DM with neuroprotection in ALS, demonstrating that people who have T2DM have a significantly lower chance of developing ALS and a delayed disease onset (Kioumourtzoglou et al., 2015; Mariosa et al., 2015; D'Ovidio et al., 2018; Tsai et al., 2019; Zeng et al., 2019). It has been hypothesised the protective role of diabetes is related to electrolyte regulation (Ahn et al., 2017). Patients suffering from diabetes often develop electrolyte disorders that cause depletion in key electrolytes including calcium. Lower calcium levels would reduce the speed of Ca^{2+} build up in neurons reducing Ca^{2+} influx into mitochondrial cells thus preserving mitochondrial function for longer (Ahn et al., 2017) one of the key pathologies observed in ALS. However, as T2DM has been shown to lead to an increase in serum ADA levels (Hoshino et al., 1994; Kurtul et al., 2004; Lee et al., 2011; Niraula et al., 2018), it is possible that the neuroprotection provided by T2DM in ALS is partially due to alterations in purine metabolism via increased ADA activity and further study could therefore investigate the relationship between ADA in T2DM, likelihood of developing ALS and disease duration.

Conclusion

ADA is a crucial enzyme in the brain as evidenced by its involvement in several neurological disorders, although the exact mechanism behind ADA dysfunction leading to the neurological impairments caused by its aberrant regulation is poorly characterised. Further study in this area should focus on the mechanisms that lead to aberrant regulation of ADA, the relationship between changes in ADA levels and adenosine and deoxyadenosine levels, and how these correlate to cellular processes that ADA, adenosine and deoxyadenosine are involved in.

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