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The key role of the NAD biosynthetic enzyme nicotinamide mononucleotide adenylyltransferase in regulating cell functions

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Abstract

The enzyme nicotinamide mononucleotide adenylyltransferase (NMNAT) catalyzes a reaction central to all known NAD biosynthetic routes. In mammals, three isoforms with distinct molecular and catalytic properties, different subcellular and tissue distribution have been characterized. Each isoform is essential for cell survival, with a critical role in modulating NAD levels in a compartment-specific manner. Each isoform supplies NAD to specific NAD-dependent enzymes, thus regulating their activity with impact on several biological processes, including DNA repair, proteostasis, cell differentiation, and neuronal maintenance. The nuclear NMNAT1 and the cytoplasmic NMNAT2 are also emerging as relevant targets in specific types of cancers and NMNAT2 has a key role in the activation of antineoplastic compounds. This review recapitulates the biochemical properties of the three isoforms and focuses on recent advances on their protective function, involvement in human diseases and role as druggable targets.

KEYWORDS

chaperones, coenzymes, enzymology, neurodegenerative disorders, NAD biosynthesis, NMNAT

Abbreviations: AD, Alzheimer's disease; AML, acute myeloid leukemia; ART, ADP ribosyltransferase; CD38, cluster of differentiation 38; CREs, cAMP-response elements; CREB, CRE-binding protein; FADS, fetal akinesia deformation sequence; HD, Huntington's disease; IMPH, IMP dehydrogenase; MAPK, mitogen-activated protein kinase; NA, nicotinic acid; NAAD, nicotinate adenine dinucleotide; NAADP, nicotinate adenine dinucleotide phosphate; NADS, NAD synthase; NAM, nicotinamide; NAMN, nicotinate mononucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NAPRT, nicotinate phosphoribosyltransferase; NGD, nicotinamide guanine dinucleotide; NHD, nicotinamide hypoxhantine dinucleotide; NMN, nicotinamide mononucleotide; NMNAT, nicotinamide mononucleotide adenylyltransferase; NR, nicotinamide riboside; NRK, nicotinamide riboside kinase; PARP, poly ADP-ribose polymerase; pTau, hyper-phosphorylated Tau; QA, quinolinic acid; QAPRT, quinolinate phosphoribosyltransferase; SARM1, sterile-alpha and TIR motif containing 1; TAD, thiazole-4-carboxiamide adenine dinucleotide; VAD, vacor adenine dinucleotide.

1 | INTRODUCTION

The importance of NAD in cellular physiology is related to its pivotal role in energy metabolism as a redox coenzyme for hundreds dehydrogenases and to its function as a co-substrate of several enzymes regulating a wide range of cellular processes. Sirtuins, ARTs, CD38, and the SARM1 protein are all NAD consumers, with significant roles in signaling, transcriptional regulation, maintenance of genome integrity, and control of the immune response, among others.[1] Sirtuins catalyze the NAD-dependent deacetylation of target substrates, like metabolic enzymes or transcription factors, thus regulating their activity. ARTs transfer the ADP ribose moiety of NAD, either as a single molecule or as a polymer, to proteins or DNA thus affecting their function. CD38 and SARM1 are NAD glycohydrolases that generate potent intracellular calcium mobilizers. By catalyzing their reactions, these enzymes consume NAD, thus making essential the continuous regeneration of the molecule. Indeed, maintenance of intracellular NAD levels is crucial for the cell and impairment of NAD homeostasis has immediate effects on the activity of these NAD-consuming enzymes, with strong implications in health and disease.[2] Altered NAD levels are linked to various pathological conditions, and boosting NAD has proven to be beneficial in preclinical models of metabolic disorders, as well as muscular and neurodegenerative diseases.[3]

NAD biosynthesis is guaranteed by the occurrence of several metabolic routes that might be operative in different combinations and with different efficiency, depending on the cell-type and metabolic status[4] (Figure 1). In this complex NAD biosynthetic network, the enzyme NMNAT catalyzes the reaction common to all routes, and therefore its activity is essential to ensure a physiological NAD homeostasis. The enzyme has been deeply characterized in its catalytic and structural properties from several organisms,[5–8] and in the last decade much progress has been made in delineating the role of the mammalian enzyme in various physiological and pathological processes. In this review, we present the current state of knowledge on the human enzyme with special focus on the most recent findings on its physiological role and influence on health and disease.

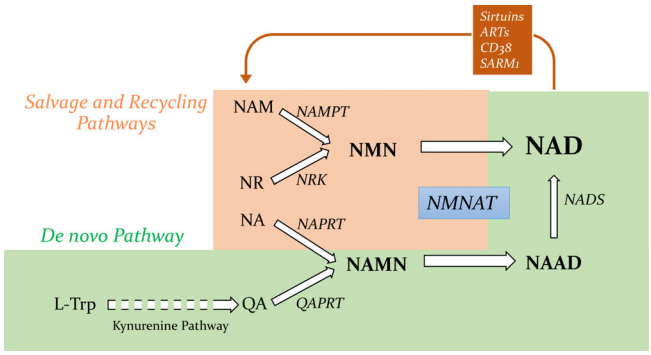

FIGURE 1 Schematic overview of NAD biosynthetic routes in mammals. The de novo pathway starts from the amino acid tryptophan that is first converted to QA through the kynurenine pathway. QA is then phosphoribosylated to NAMN by QAPRT and NAMN is converted to NAD by two consecutive reactions catalyzed by NMNAT and NADS. NMNAT adenylates NAMN to the corresponding dinucleotide NAAD and NADS amidates NAAD to NAD. NAD can also be salvaged from the three forms of vitamin B3, that is, NA, NAM, and NR. In detail, NA enters the de novo pathway after being converted to NAMN by NAPRT. NR, and NAM are first transformed into NMN by NRK and NAMPT, respectively, and NMN is finally adenylated to NAD by NMNAT. The sequential action of NAMPT and NMNAT also recycles back to NAD the NAM produced by the activity of the NAD-consuming enzymes. The enzyme NMNAT can use both NMN and NAMN as substrates, and therefore it is common to the de novo pathway and all salvaging and recycling routes

2 | CATALYTIC AND STRUCTURAL PROPERTIES OF NMNAT ISOFORMS

NMNAT (EC 2.7.7.1.) catalyzes the transfer of the adenylyl moiety of ATP to NAMN or NMN yielding NAAD or NAD, respectively, and releasing pyrophosphate. The reaction is reversible with a K_{eq} of about 0.3, as calculated at pH 8.5, at room temperature.[9] In mammals, three NMNAT isoforms have been described, deriving from distinct genes and exhibiting different oligomeric structures, catalytic properties, and tissue distribution. They also have distinct intracellular localizations as shown in Figure 2. NMNAT1 is the nuclear isoform. It is the most abundant among the isoforms and it is ubiquitously expressed.[17,18] It is also the most catalytically efficient and it is about four-times more specific for NMN than NAMN.[9,19] NMNAT2 is associated to the cytosolic surface of the Golgi apparatus.[20,21] Its presence is limited to a few tissues, including brain, heart, skeletal muscle, and pancreas.[9,22,23] It is the least efficient among the three isoforms and uses NAMN and NMN with comparable efficiencies.[19] NMNAT3 is present in the cytoplasm, in the mitochondrial matrix and inside lysosomes.[9,16,24–26] It is generally less abundant than NMNAT1 and restricted to some tissues, including lung, spleen, kidney, and placenta,[24] but it represents the major isoform in erythrocytes.[27] The recombinant enzyme uses NMN and NAMN with the same efficiency.[19] Whether this isoform would exhibit different

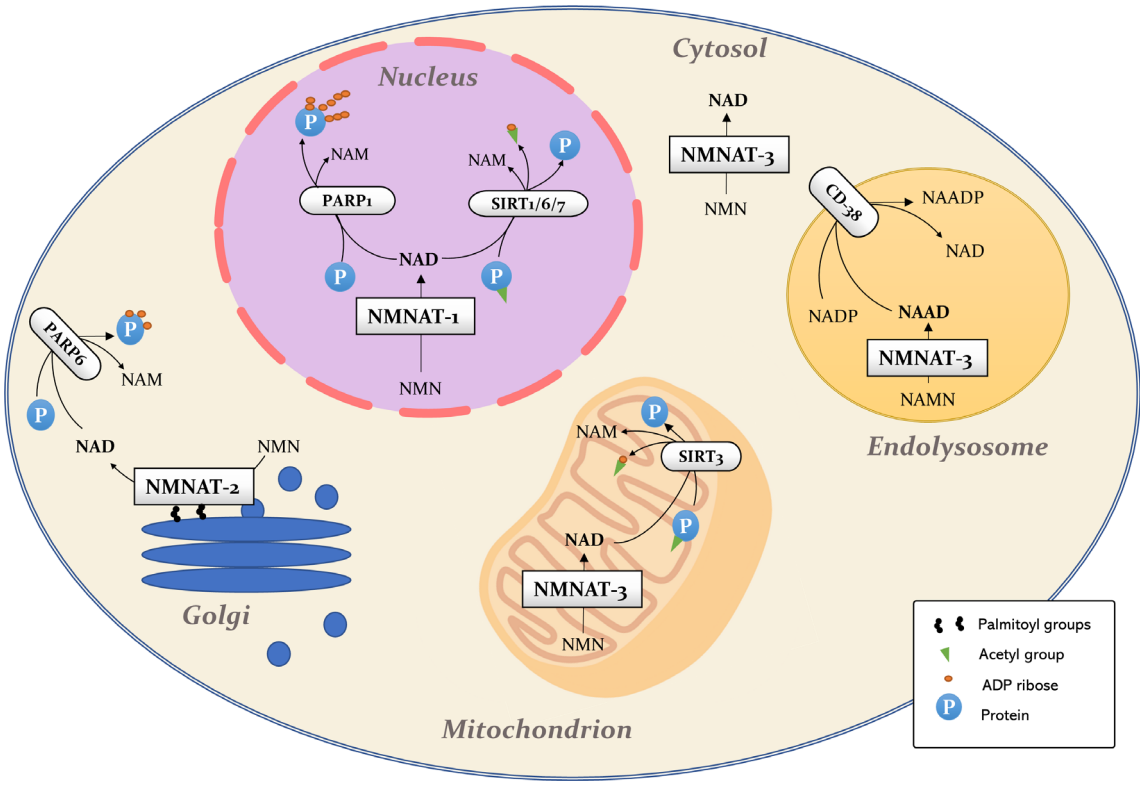

FIGURE 2 Subcellular compartmentalization of NMNAT isoforms. The scheme shows the subcellular compartmentalization of the three isoforms highlighting their known functional interactions with specific NAD-consuming enzymes. In the nucleus, NMNAT1 provides NAD to sirtuins and PARP1 regulating their activities.[10–12] NMNAT2 is anchored to the membranes of the Golgi apparatus and contributes to maintain NAD levels in the cytosol where it functionally interacts with PARP6.[13] Within mitochondria, NMNAT3 provides NAD to mitochondrial ARTs[14] and SIRT3,[15] whereas in endolysosomes it contributes with CD38 to NAADP generation[16]

substrate specificity depending on its subcellular localization has not been investigated.

The three isoforms exhibit similar kinetic parameters, with K_m values for the substrates in the micromolar range.[7,19] They show a variable specificity toward ATP analogs. In particular, NMNAT3 uses very well GTP or ITP to synthetize the corresponding pyridine dinucleotides NGD and NHD.[9,28] Indeed, levels of these dinucleotides were found to significantly increase in NMNAT3 overexpressing mice.[29] NMNAT3 is also the most efficient in adenylating reduced NMNH to NADH,[9,19] an activity which is required in vivo for the NAD boosting effect of exogenously administered reduced NRH or NMNH, which are emerging as NAD precursors much more effective than NR or NMN.[30,31] The difference in substrate specificity and metal–ion requirement of the three isoforms has been exploited to develop a biochemical discrimination assay that can measure each isoform-specific activity in mice tissue extracts.[28]

The 3D structures of human NMNAT1 and NMNAT3 have been solved in apo-form and in complex with substrates or products.[24,32–34] Monomers of the two isoforms are very similar, sharing a central core with the typical Rossmann fold and a similar active site arrangement. However they adopt different oligomeric states, namely hexameric for NMNAT1 and tetrameric for NMNAT3 (Figure 3). They also show two highly disordered, isoform-specific regions, of about 40 and 20 residues, respectively, that comprise the subcellular localization signals. However, while in NMNAT1 such a region is required for the nuclear localization, in NMNAT3 it seems to be dispensable for mitochondria targeting.[20] Resolution of the 3D structure of the enzyme gave a rationale for the NMNATs' dual specificity toward NAMN and NMN, which is mainly due to several key water molecules in the active site that allows accommodation of substrates with different electrostatic properties, without the need for significant conformational changes.[33] This binding flexibility makes NMNATs versatile in intercepting both NMN- and NAMN-metabolic fluxes for NAD formation. Therefore, the contribution of the amidated and deamidated pathways to overall NAD biosynthesis is dictated by the expression of the enzymes upstream of NMNAT, namely NRK, NAMPT, QAPRT and NAPRT (Figure 1).[35] The 3D structure of NMNAT2 has not been solved yet. Structural models have been

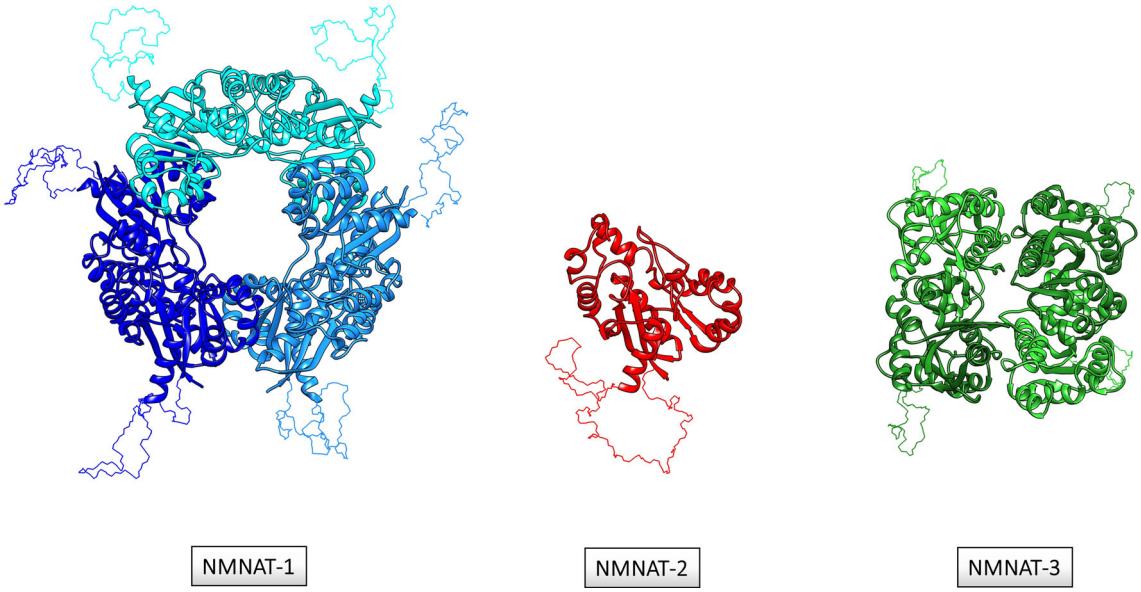

FIGURE 3 Structural representation of human NMNAT isoforms. The crystal structures of NMNAT1 (PDB ID: 1KQN) and NMNAT3 (PDB ID: 1NUR), and the homology model of NMNAT2 (obtained with SWISS MODEL online server, by using 1KQN as the template) are illustrated as ribbon diagrams. The disordered isoform-specific regions have been modeled with SWISS MODEL and are indicated as backbone chain traces

generated that suggest a topology highly similar to the other isoforms and revealed an isoform-specific domain of about 60 residues, which is dispensable for the catalytic activity and essential for anchoring the enzyme to the Golgi membrane via palmitoylation of two adjacent cysteine residues.[20,21,36] In dendrites and axons, such a domain is required for the enzyme's association with Golgi-derived axonal transport vesicles.[21,37]

3 | REGULATION OF NMNAT ISOFORMS

The regulation of the three isoforms at the transcriptional and post-transcriptional level has been poorly investigated, with studies limited to the NMNAT2 isoform. The *NMNAT2* gene promoter contains two CREs and evidence of gene regulation via CREB signaling has been provided.[38] Indeed, the impairment of the CREB-regulated transcription observed in the brains of a mice model of tauopathy was found to account for the reduced expression of NMNAT2.[38] Also, two putative responsive elements have been identified within the first intron of the *NMNAT2* gene, which are recognized by p53. In particular, binding of p53 to these elements upon DNA damage activates gene transcription.[39] NMNAT2 is the most labile among the isoforms, with a very short half-life, and degradation occurring, at least in part, via the ubiquitin proteasome system.[40,41] Due to its extreme liability, neurons, in particular, require continuous synthesis of new protein and constant axonal transport. Indeed, upon neuron injury the isoform is rapidly degraded and its loss markedly contributes to axon degeneration.[40] Accordingly, factors that slow the protein degradation also delay axon degeneration after the injury.[37,42,43] Interestingly, dissociation of the protein from the axonal transport vesicles increases its half-life, which suggests a role of palmitoylation and membrane attachment in the protein turnover.[44] The MAPK signaling pathway, whose activation is required for axon degeneration, seems also to control the NMNAT2 protein stability in neurons, as deletion of some pathway's components increases the level of the endogenous protein.[45,46]

A high-throughput screening platform developed to detect endogenous NMNAT2 levels in cortical neurons allowed identification of several positive and negative regulators exerting significant impact on the enzyme's levels when administered to mice in the μM range.[47] Among the positive regulators are caffein and aspartic acid, whereas among the negative ones is retinoic acid. The nature of these compounds suggests that neuronal NMNAT2 levels can be upregulated via enhanced excitatory neurotransmission and upon increase in cAMP, which is in keeping with the presence of CREB binding sites on the NMNAT2 promoter.[46]

Much less is known on the regulation of NMNAT1. The enzyme has been found to be phosphorylated in vivo, with the phosphorylation sites residing in the loop that dictates the nuclear localization. In particular,

phosphorylation of Ser136 by protein kinase C was found to prevent the enzyme interaction with PARP1.[48] To our knowledge, no studies have been reported so far on the regulation of NMNAT3.

4 | NMNATS FUNCTION IN HEALTH AND DISEASE

4.1 | Nonredundant functions for NMNAT isoforms

Although the same cell can use three different isoenzymes for NAD biosynthesis, each isoform is essential for survival. In fact, the homozygous deletion of the individual genes in mice is lethal, indicating that the three isoforms cannot compensate each other. In particular, NMNAT1 and NMNAT3 KO mice do not survive to birth[49] and die postnatally from anemia,[50] respectively, whereas homozygous NMNAT2 mutant mice die perinatally, showing a greatly distended bladder, underdeveloped diaphragm and a reduction in total skeletal muscle mass.[51] Also, overexpression of NMNAT1 in the nucleus does not block induced nerve damage in an NMNAT2 deficient context, but its overexpression in the cytosol is neuroprotective.[52,53] Likewise, depletion of NMNAT2 decreases cytoplasmatic, but not nuclear NAD concentrations, contrary to the expectation that NAD might freely cross the nuclear membrane.[54] Therefore, nuclear and cytosolic NAD concentrations, which in cultured mammalian cells range from 60 to 260 μM,[54,55] are locally and specifically regulated by NMNAT1 and NMNAT2, respectively, in a compartment-specific manner. Moreover, the two isoforms can affect each other's activity: in fact, stimulation of NMNAT2 during the early stage of adipogenesis increases cytoplasmic NAD, at the same time depleting NMN availability. As a consequence, NMNAT1 activity decreases leading to decrease NAD levels in the nucleus with the consequent suppression of PARP1 activity, which drives differentiation of precursor cells into adipocytes.[56]

The contribution of NMNAT3 to NAD biosynthesis is less studied. The isoform has a critical role in the maintenance of the NAD pool in mature erythrocytes, and loss of its gene impairs glycolysis causing hemolytic anemia.[50] However, its contribution to NAD biosynthesis in mitochondria where NAD concentration ranges from 300 to 500 μM[54,57] is matter of debate. Some authors showed that NMNAT3 is dispensable for mitochondrial NAD maintenance,[58,59] whereas others evidenced an important role in regulating mitochondrial NAD levels[15,29] and mitochondrial mono ADP-ribosylation.[14] A recent study showed that inside endolysosomes, NMNAT3 is responsible, together with CD38, of the production of NAADP, a potent Ca^{+2}-mobilizing second messenger. In fact, in these organelles, CD38 catalyzes NAADP synthesis by exchanging the nicotinamide moiety of NADP with the NA group of NAAD which is locally produced by NMNAT3[16] (Figure 2).

4.2 | NMNATs cross-talk with NAD-dependent enzymes

By catalyzing a key reaction in the NAD biosynthetic pathway, NMNAT is essential to support the catalytic activity of the NAD consuming enzymes. Therefore NMNAT levels are expected to play a significant role in regulating the activity of these enzymes. This has been clearly demonstrated in cultured cells where *NMNAT1* silencing increases the acetylation level of p53 by impairing the deacetylating activity of NAD-dependent sirtuins.[10,60] Also, a functional interaction has been shown to occur between NMNAT2 and PARP6 in the cytosol of ovarian cancer cells (Figure 2). In these cells, the NAD produced by NMNAT2 is essential for the PARP6-dependent mono ADP-ribosylation of ribosomal proteins that maintains proteostasis to support cell growth.[13] Furthermore, in injured neurons, NMNAT2 markedly affects the functional properties of SARM1, as discussed in more detail in the next section.

The marked dependence of the NAD-consuming enzymes from NMNAT activity is also evident from the physical interaction that NMNAT1 establishes with PARP1 and SIRT1, as demonstrated through immunoprecipitation experiments.[11,12] Such interaction occurs at the promoter of the genes controlled by the NAD-dependent enzymes and results in a more efficient NAD utilization through substrate channeling. Activation of PARP1 by NMNAT1 has been documented to occur also independently of NAD production, possibly via an allosteric mechanism.[11,48] In its unphosphorylated form, NMNAT1 is in fact able to bind to the ADPR polymers of auto ADP-ribosylated PARP1, thus stimulating its activity.[48] A physical interaction has been reported also to occur between NMNAT3 and SIRT3 in mitochondria, with NMNAT3 providing NAD to SIRT3, and SIRT3 deacetylating NMNAT3 thus enhancing its enzymatic activity[15] (Figure 2).

4.3 | The dual protective role of NMNATs: NAD synthesis and chaperone-like activity

NMNAT exerts a protective effect in several physiological and pathological conditions. Most of such effects are due

to its enzymatic activity which fuels the NAD-dependent deacetylase activity of sirtuins. Both *NMNAT1* and *NMNAT2* genes are upregulated upon exposure to DNA damaging agents and their activation sustains the DNA-damage response in cultured cells.[39,60,61] NMNAT1 plays an important role also in cell survival during nutrient deprivation, when cells require downregulation of ribosomal biogenesis to reduce protein translation and save energy. In fact, knockdown of the gene in HeLa cells prevents the down-regulation of ribosomal RNA synthesis after glucose starvation.[60] NMNAT2 and NMNAT3 activities protect rat cardiomyocytes from angiotensin II-induced hypertrophy,[15,62] and NMNAT2 is also critical during oocytes maturation in mice, as in aged oocytes a marked decrease in the enzyme expression reduces NAD levels and induces metabolic dysfunctions and meiotic defects.[63]

Significant progress has been made in exploring the protective effect exerted by NMNAT2 in the nervous system. The discovery that NMNAT2 is a critical survival factor for axons originated from the observation that in a mouse strain (Wallerian degeneration slow mice), transected axons survive much longer than normal thanks to the presence of a cytosolic aberrant protein endowed with NMNAT activity.[64] Subsequent studies demonstrated that axon maintenance relies on the presence of a functional NMNAT2, as depletion or impairment of the catalytic activity of this isoform causes spontaneous neurite degeneration that cannot be prevented by the other isoforms.[40,65] In keeping with a role in axon maintenance, reduced levels of NMNAT2, both as transcript and protein, have been reported in the brains of a mice model of human tauopathy prior the onset of the cognitive defects, and overexpression of the enzyme in the animals markedly reduced neurodegeneration.[38] In human brain, NMNAT2 transcript levels correlate positively with global cognitive function and negatively with AD pathology.[66] Recently, it has been established that NMNAT2 protects neurons from axon degeneration by blocking SARM1, a primary regulator of axon auto-destruction upon injury.[67] In particular, the increase in NMN and the decrease in NAD, which are secondary to NMNAT2 loss upon injury, have been suggested to trigger the intrinsic NAD hydrolase activity of the SARM1 TIR domain, which in turn drives axon destruction.[68–72]

Notably, the neuroprotective effect of NMNAT2 seems also to be mediated by the protein ability to act as a molecular chaperon, independently of the NAD biosynthetic activity. The chaperone-like function has been first described for NMNAT from *Drosophila* and human NMNAT3.[73] Authors demonstrated the ability of these NMNATs to protect proteins from unfolding and to promote refolding both in vivo and in vitro. However, the molecular mechanism underlying the holdase and foldase activities remains unknown. In subsequent studies, starting from the evidence that NMNAT2 colocalizes with Hsp90 and hyper-phosphorylated Tau (pTau) in the insoluble fractions of brains from AD patients,[66] authors verified the chaperone-like activity of NMNAT isoforms against the aggregation of pTau by using the recombinant proteins, demonstrating that such activity is conserved in all three isoforms.[74] In particular, a physical interaction between NMNAT3 and the phosphorylated sites of pTau has been demonstrated to occur, which would explain the protection against pTau aggregation. Moreover, NMNAT3 was found to mediate binding of pTau to Hsp90, indicating that it might act as a co-chaperone to assist Hsp90 in the clearance of pTau.[74] These results are in keeping with the ability of the different NMNAT isoforms to reduce the abnormal aggregation and cytotoxicity of pTau in different models of neurodegenerative diseases.[38,73,75] The NMNAT chaperon-like activity has a protective role also in HD. In a fly model of HD, overexpression of NMNAT in brains or neurons reduces the aggregation of mutant huntingtin by directly interacting with the aggregates and facilitating their autophagic clearance, thus restoring neuronal function.[76] All together, these studies highlight the therapeutic potential of NMNAT in various proteinophaties.

4.4 | NMNATs in human genetic diseases

Multiple mutations in the *NMNAT1* genes are associated with Leber congenital amaurosis 9, a severe blinding retinal disease.[77–80] The biochemical characterization of the NMNAT1 variants indicated that the disease likely arises from a combination of reduced catalytic activity and decreased protein stability.[81] The reason why NMNAT1 mutations causes a pathology confined to the retina is still matter of investigation. Recent studies in a mice model of NMNAT1 associated retinal degeneration have highlighted an overactivation of PARP1 and a consequent drop in NAD levels specifically in retina and not in other tissues.[82]

A homozygous missense mutation in the *NMNAT2* gene has been reported to be associated with a childhood-onset polyneuropathy with erythromelalgia.[83] Notably, the mutation impairs both the activity and thermal stability of the enzyme, and increases its turnover rate in cells. A more severe and lethal phenotype (FADS) is associated with heterozygous mutations that, again, impact both the enzymatic activity and protein stability.[84] Given the critical role of NMNAT2 in axon survival, it is evident that the enzyme mutations underlie the

neuropathy and the compromised neuronal development that contribute to FADS.

A single-nucleotide polymorphism located 126 kb downstream of the *NMNAT3* gene has been identified in a dutch cohort of familial late-onset AD, suggesting that this isoform might be relevant to AD.[85]

4.5 | NMNATs in aging

Aging is characterized by a markedly decrease of NAD levels across multiple tissues, and it is now widely accepted that such a decline contributes to all its traits.[86] The decrease in NAD level is caused by a severe impairment in NAD homeostasis, due to the overactivation of NAD consuming enzymes, like PARP1 and CD38, and the concomitant decrease of NAD biosynthesis. Much interest has been devoted to the age-related down-regulation of the biosynthetic enzyme NAMPT,[87] whereas contribution of NMNAT in NAD decline has been poorly investigated. Overexpression of NMNAT in *Drosophila* was found to extend lifespan by improving oxidative stress response and mitochondrial function.[88] However, studies on the mammalian isoforms are lacking. Unexpectedly, NMNAT2 expression in rat hearts is reported to markedly increase with age, while the other two isoforms do not show significant age-related changes.[41]

4.6 | NMNATs in cancer

The first interest on NMNAT in cancer arose from the discovery of its role in the activation of the prodrug tiazofurin for cancer chemotherapy. In fact, the enzyme catalyzes adenylation of tiazofurin 5′-monophosphate to the active metabolite TAD, which is a potent inhibitor of IMPH.[19] Inhibition of IMPH results in impaired synthesis of guanylic nucleotides, with consequent cell death. Resistance to tiazofurin exhibited by some cancer cells was found to be related to the impairment of TAD biosynthesis caused by reduced NMNAT levels.[89] The finding that overexpression of NMNAT2, but not of the other isoforms, increased tiazofurin sensitivity in a colorectal cancer cell line suggested that the NMNAT2 isoform was responsible of the activation of the pro-drug in vivo.[90] Notably NMNAT2 is also the isoform involved in the activation of vacor, an old rat poison shown to be cytotoxic against NMNAT2-expressing cells.[91] Vacor is in fact converted into VAD by the consecutive action of NAMPT and NMNAT2. Once formed, VAD impairs the activity of NAD-dependent dehydrogenases, triggering necrosis in cancer cells and tumor xenografts expressing NMNAT2, like melanoma and neuroblastoma.[91] Altogether, these finding envisage a role of NMNAT2 in different toxification routes to generate pyridine antimetabolites for antitumor therapy.

Although it has been established that cancer cells have a higher demand of NAD than normal cells, the investigation of NAD biosynthetic enzymes as direct anticancer targets has been limited to only a few of them. Much effort has been devoted to the development of inhibitors of the enzyme NAMPT, and most of the identified compounds have shown promising antitumoral activity in pre-clinical studies. However none has so far progressed in later clinical stages, mainly for toxicity problems.[92] An additional issue in targeting NAMPT is that NAD precursors present in our diet can rescue the antineoplastic effect of its inhibition. NAMPT requirement for NAD biosynthesis can in fact easily by-passed as cells can shift to alternative biosynthetic routes (Figure 1). In this view, NMNAT, for its ability to catalyze the reaction common to all routes, might represent an anticancer target worth to be explored. However, to date only limited studies have addressed this issue.

NMNAT1 gene is located in a chromosomal region that undergoes heterozygous deletion in about 20% of several human tumor types (lung, renal and colorectal cancers), leading to a reduced expression of the enzyme at both transcript and protein level.[60] It has been speculated that since NMNAT1 contributes to the suppression of rRNA transcription and tumor cells have high levels of ribosomal biogenesis, reduced NMNAT1 expression may facilitate tumor development.[60] On the other hand, low NMNAT1 expression was found to correlate with better survival of patients with sarcomas, liver hepatocellular carcinoma, bladder carcinoma, breast cancer, esophageal adenocarcinoma, kidney renal papillary cell carcinoma, pancreatic ductal adenocarcinoma and uterine corpus endometrial carcinoma, indicating that the enzyme might be important for the tumor progression.[61] Also, in a breast cancer cell line a decrease in the expression of NMNAT1 is accompanied by a reduction of NAD which decreases poly ADP-ribosylation of the multifunctional nuclear protein CCCTC-binding factor, leading to epigenetic silencing of tumor suppressor genes.[93] In keeping with a role of NMNAT1 in tumor development, a very recent study identified NMNAT1 essential in maintaining NAD levels for AML progression and chemoresistance.[10] Authors demonstrated that NMNAT1 deletion in AML cells blocks cell cycle and causes apoptosis, effects which are mediated by p53 activation. Indeed NMNAT1 fuels NAD to SIRT6 and SIRT7 which deacetylate, and hence inactivate, p53. Importantly, while leukemia stem cells depend on the catalytic activity NMNAT1 for their maintenance, normal hematopoiesis and hematopoietic stem

cells do not depend on NMNAT1. As expected, NAD precursors that might be available in physiological settings were not able to rescue the dependency of AML cells on NMNAT1. Experiments in mice confirmed the NMNAT1 requirement for leukemogenesis, so identifying NMNAT1 as a promising therapeutic target for AML.[10]

Recent studies report on NMNAT2 deregulation in cancer and provide evidence of its involvement in tumor progression. In particular, NMNAT2 levels increase in colorectal cancer, with a positive correlation with tumor invasiveness and stage.[94] Higher levels of this isoform are also detected in ovarian cancer cells.[13] Here, NMNAT2 was found to support the activity of PARP16, which by mono ADP-ribosylating ribosomal proteins maintains proteostasis during accelerated cell proliferation. Indeed, deletion of NMNAT2 promotes protein aggregation, reducing the growth of cancer cells.[13]

Although the available findings clearly point to NMNAT2 and NMNAT1 as very promising targets in specific cancer types, only a few enzymes' inhibitors have been identified and characterized so far.[95] Gallotannin, a polyphenolic plant metabolite, inhibits all three isoforms, with NMNAT3 being the most sensitive (IC50 2 μM).[9] Nucleotide polyphosphates, namely nicotinamide/nicotinate-riboside-P*n*-adenosine (Np3AD, Np4AD and NAp4AD) showed selective inhibition against the different isoforms, although with IC50 in the micromolar range.[19] Recently, a weak inhibition of NMNAT2 was found to be exerted by the NAD analog VAD.[91]

5 | CONCLUSIONS

Although the first evidence of mammalian NMNAT dates back to 1948,[96] and the human enzyme has been characterized in its molecular and catalytic properties about two decades ago, its key role in various physiological and pathological processes has been addressed only recently, and several aspects of its function in cellular biology still remain unexplored. In particular, our knowledge on the enzyme's regulation at transcriptional and translational levels is very limited. The occurrence of three distinct isoforms, with different subcellular localization, that modulate NAD levels in different cellular compartments, suggests that the three isoforms might be differentially regulated, adding complexity to their study. Likewise, it remains to be clarified the contribution of each isoform to NAD biosynthesis in different physiological and pathological conditions. Very limited are for example the studies on the contribution of each isoform to the NAD decline observed during aging. On the other hand, significant progress has been made in exploring NMNAT2 as an important enzyme for mammalian brain health, and it is now clear that maintenance of its activity and levels may serve as a therapy to protect against neurodegeneration. Important data on the role of NMNAT1 and NMNAT2 in cancer development and progression are also emerging, which should drive future studies in the design and improvement of anticancer pro-drugs that might be activated by NMNATs, as well as in the development of specific enzyme's inhibitors for new therapeutic strategies.

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