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The function of nicotinamide phosphoribosyltransferase in the heart

Chiao-Po Hsu², Takanobu Yamamoto¹, Shinichi Oka¹, and Junichi Sadoshima^{1,3}

¹Cardiovascular Research Institute, Department of Cell Biology and Molecular Medicine, Rutgers–New Jersey Medical School, Newark, New Jersey, USA

²Division of Cardiovascular Surgery, Department of Surgery, Taipei Veterans General Hospital, National Yang-Ming University School of Medicine, Taipei, Taiwan, Republic of China

Abstract

In addition to its roles as a coenzyme and an electron transfer molecule, nicotinamide adenine dinucleotide (NAD⁺) has emerged as a substrate of sirtuins, a family of enzymes that control aging and metabolism. Nicotinamide phosphoribosyltransferase (Nampt), a rate-limiting enzyme in the NAD⁺ salvage pathway, plays an important role in controlling the level of NAD⁺ and the activity of Sirt1 in the heart and the cardiomyocytes therein. Nampt protects the heart from ischemia and reperfusion injury by stimulating Sirt1. In this review, we summarize what is currently known regarding the function of Nampt in the heart.

Introduction

Nicotinamide adenine dinucleotide (NAD⁺) is classically regarded as a coenzyme for energy production and participates in cellular redox reactions as a transfer molecule for electrons. During the past decade, NAD⁺ was also found to serve as a substrate of sirtuins and activated poly(ADP-ribose) polymerase (PARP).[1, 2] Sirtuins are involved in gene silencing, longevity, senescence, differentiation, and cell survival.[3,4] The nuclear enzyme PARP-1 is involved in DNA repair and maintenance of genomic stability in response to DNA damage.[1]

In mammals, NAD⁺ is synthesized from amino acids, including tryptophan and aspartic acid, via the de novo pathway,[5] or is taken up from the extracellular space.[6, 7] NAD⁺ can also be resynthesized from NAD⁺ metabolites through the salvage pathway.[5] In the salvage pathway of NAD⁺ biosynthesis, Nampt is identified as a rate-limiting enzyme that converts nicotinamide to nicotinamide mononucleotide (NMN).[8, 9] NAD⁺ is then

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³Address correspondence to: Junichi Sadoshima, Cardiovascular Research Institute, Rutgers–New Jersey Medical School, 185 South Orange Avenue, Medical Science Building G-609, Newark, New Jersey, 07103, USA. Phone: (973) 972-8619; Fax: (973) 972-8919. Sadoshju@njms.rutgers.edu.

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synthesized from NMN by NMN adenylyltransferase (Nmnat).[10, 11] Nampt has received attention recently not only because it can increase the intracellular NAD⁺ level, but also because it is a mammalian functional equivalent of pyrazinamidase/nicotinamidase 1 (PNC1), a master gene that contributes to longevity in yeast. [12]

Nampt

Nampt is a highly conserved 52-kDa protein without a signal sequence,[12, 13] but it exists in most cells and tissues.[12] Nampt is found in both intracellular and extracellular spaces. Intracellular Nampt (iNampt) is primarily involved in NAD⁺ synthesis, whereas extracellular Nampt (eNampt), secreted from some cell types including neutrophils, adipocytes, cardiomyocytes and mesangial cells, may act as an extracellular NMN synthesis enzyme or as a cytokine/adipokine.[12, 14–17] The mechanism through which Nampt is secreted is not well understood. However, Nampt may leak from damaged tissues, which may explain why Nampt serum levels increase in a mouse model of acute myocardial infarction.[18]

The gene encoding Nampt/PBEF was isolated from a human peripheral blood lymphocyte cDNA library in 1994. It was named pre-B-cell colony-enhancing factor (PBEF) because it was thought to be a cytokine that acted on early B-lineage precursor cells.[12] Later, on the basis of sequence similarity between PBEF and nadV, a prokaryotic nicotinamide phosphoribosyltransferase (NAmPRTase) from *Haemophilus ducreyi*, PBEF was identified as Nampt, an enzyme involved in NAD⁺ biosynthesis.[9, 19] Recently, visfatin, an adipocytokine proposed to regulate insulin secretion in pancreatic β -cells, was found to be identical to Nampt,[17] although cytokine actions of Nampt require further confirmation. Nampt might be involved in aging and a broad range of disorders, including human immunodeficiency virus infection, septicemia, myocardial failure, atherosclerosis, metabolic disorders, inflammatory diseases, malignancies, and neurodegenerative disorders, through both NAD⁺ synthesis and cytokine actions.[20]

Nampt controls NAD⁺ levels in the heart

Downregulation of Nampt significantly decreases NAD⁺ content in cardiomyocytes at baseline, suggesting that Nampt plays a crucial role in NAD⁺ synthesis in cardiomyocytes. [21] On the other hand, overexpression of Nampt readily increases NAD⁺ content, indicating that Nampt is a rate-limiting enzyme for NAD⁺ synthesis in cardiomyocytes.[21] Interestingly, Nampt is downregulated at both the protein and mRNA levels under stress conditions in the heart, including 24 hours of ischemia, 45 minutes of ischemia followed by 24 hours of reperfusion, and 2 and 4 weeks of pressure overload due to thoracic aortic constriction,[21] which induce compensated and decompensated stages of cardiac hypertrophy, respectively, in mice. These changes are accompanied by decreases in the level of NAD⁺ in the heart ([22] and our unpublished observation). These results suggest that Nampt is a critical determinant of the NAD⁺ level in the heart at baseline and in response to stress.[23, 24]

Generation of transgenic mice with cardiac-specific overexpression of Nampt

In order to evaluate the function of Nampt in the heart, transgenic mice with cardiac-specific overexpression of Nampt (Tg-Nampt) were generated. These mice exhibited higher levels of NAD⁺ and NAD⁺/NADH than non-transgenic (NTg) control mice at baseline. The baseline cardiac phenotype, including heart weight, chamber size, and left ventricular function, was not significantly different between Tg-Nampt and NTg mice.[21] This observation is different from a recent report by Pillai et al.,[14] in which mice with Nampt overexpression developed cardiac hypertrophy. The reason for the discrepancy remains to be elucidated but the level of Nampt overexpression was greater in Pillai's report than in ours.

Nampt protects the heart against ischemia/reperfusion (I/R) injury

To examine the effect of Nampt upon myocardial I/R injury, Tg-Nampt mice were subjected to 45 minutes of ischemia followed by 24 hours of reperfusion and the degree of myocardial infarction was evaluated. The size of the myocardial infarct/area at risk was significantly smaller in Tg-Nampt than in NTg mice.[21] Since endogenous Nampt is downregulated by I/R and cardiac-specific overexpression of Nampt restores the NAD⁺ level,[21] these results suggest that downregulation of endogenous Nampt contributes to myocardial injury in response to I/R and that upregulation of Nampt protects the heart against I/R injury. The number of TUNEL-positive cells in the ischemic border zone was significantly lower in Tg-Nampt mice than in NTg mice, suggesting that Nampt suppresses cardiomyocyte apoptosis. [21] In order to examine whether Nampt has cell-autonomous and protective effects against cell death in cardiomyocytes, we transduced cardiomyocytes with adenovirus harboring Nampt or shRNA-Nampt. Overexpression of Nampt protected cardiomyocytes from cell death induced by either methylmethane sulfonate (MMS), a DNA-alkylating agent known to induce necrotic cell death, [25] or glucose deprivation. On the other hand, downregulation of Nampt significantly increased apoptotic cell death in cardiomyocytes, as evaluated with TUNEL staining and immunoblot analyses of cleaved caspase-3.[21] Furthermore, the increase in cell death induced by downregulation of Nampt was reversed in the presence of adenovirus harboring Bcl-xL, an inhibitor of apoptosis.[21] These results suggest that Nampt directly regulates apoptosis in cardiomyocytes.[21] We also investigated whether knockdown of Nampt causes necrotic cell death. Nampt downregulation alone did not significantly increase the number of necrotic myocytes, as evaluated with propidium iodide staining and *in situ* ligation with hairpin 2 probes. However, Nampt downregulation significantly increased necrotic cell death in the presence of glucose deprivation or MMS. [21] Taken altogether, these results suggest that although downregulation of Nampt induces apoptotic cell death at baseline, it may facilitate necrotic cell death under stress.

What, then, is the underlying mechanism by which Nampt protects cardiomyocytes from cell death? Upregulation of Nampt increases the cellular NAD⁺ level and enhances the activity of Sirt1 in mouse fibroblasts.[8, 26] We have shown previously that the size of the myocardial infarct and the number of TUNEL-positive nuclei induced by ischemia/reperfusion are significantly reduced in transgenic mice with cardiac-specific overexpression of Sirt1 compared to NTg mice,[27] whereas cardiac-specific Sirt1 homozygous knockout

mice exhibit significantly larger myocardial infarcts and greater numbers of apoptotic cardiomyocytes than NTg mice.[28] Thus, we speculate that Sirt1, an NAD⁺-dependent enzyme, may play a role in mediating the effect of Nampt in cardiomyocytes.[29]

Although the results described thus far suggest that cardiac-specific overexpression of Nampt protects the heart, the role of eNampt in regulating I/R injury remains unclear. Lim et al. showed that eNampt is capable of reducing myocardial injury when administered at the time of myocardial reperfusion by regulating the PI3K and MEK1/2 pathways and mitochondrial permeability transition pore (mPTP) opening.[30] Xiao et al. showed that pretreatment with eNampt attenuates apoptotic cell death and mitochondrial membrane potential depolarization in H9c2 myocardial cells in response to H₂O₂ treatment.[31] Although eNampt did not inhibit the death receptor-dependent apoptotic pathway, it suppressed the mitochondria-dependent apoptotic pathway by regulating p53 and Bcl-2 family proteins through AMPK activation.[31] Taken altogether, these results suggest that eNampt may contribute to the protective effect of Nampt against I/R observed in Tg-Nampt. Because Nampt could be released from cardiomyocytes, [14] the relative contribution of iNampt and eNampt to protection against I/R injury in Tg-Nampt remains to be elucidated. Montecucco et al. showed that inhibition of Nampt by FK866, a chemical inhibitor, decreased myocardial infarct size in a mouse model of I/R *in vivo* by reducing neutrophil-mediated injury through downregulation of CXCL2.[18] This would appear to indicate that Nampt has a detrimental effect upon I/R injury. It is unknown whether eNampt mediates the detrimental effect of Nampt. The NAD⁺ salvage pathway is regulated by the circadian clock through epigenetic control and chromatin remodeling,[32, 33] and also by AMPK in the heart.[34] Whether NAD⁺ exhibits circadian changes in the heart, and if so, whether such a mechanism contributes to the well-known circadian variation in the incidence of myocardial infarction in humans requires further investigation.

Nampt protects the heart against I/R injury in part by stimulating autophagy

Since Nampt protects the heart against ischemia, we examined whether Nampt affects autophagy, a cellular mechanism of degradation to preserve cellular ATP levels and/or to achieve quality control of proteins and organelles.[35] Nampt knockdown (Ad-shRNA-Nampt) increased autophagosomes in cardiomyocytes, as assessed by counting of GFP-LC3 puncta, which allow visualization of autophagosome formation, and by LC3-II immunoblot analyses.[21] Importantly, however, the accumulation of GFP-LC3 puncta observed in the presence of Nampt knockdown was not enhanced in the presence of chloroquine,[21] an inhibitor of autophagosome-lysosome fusion. p62/SQSTM1, a protein known to be degraded by autophagy, [36–38] was accumulated in the presence of Nampt knockdown, whereas the mRNA level of p62 was not significantly affected.[21] These results suggest that downregulation of Nampt inhibits autophagic flux in cardiomyocytes. We speculate that NAD⁺ synthesis by Nampt plays an important role in mediating either fusion between autophagosomes and lysosomes or lysosomal degradation of cargo proteins.

Supplementation with exogenous NAD⁺ significantly reversed the accumulation of autophagosomes in the presence of Nampt knockdown,[21] suggesting that the effect of Nampt downregulation is mediated through decreases in NAD⁺. Since Sirt1 stimulates

autophagy in HeLa and MEF cells through deacetylation of autophagy-related proteins,[39] we examined the effect of Sirt1 knockdown upon autophagy in cardiomyocytes. Downregulation of Sirt1 caused accumulation of LC3-II and p62, thereby mimicking the effect of Nampt downregulation.[21] Furthermore, double knockdown of Nampt and Sirt1 did not show an additive effect upon accumulation of LC3-II and p62,[21] suggesting that Nampt and Sirt1 affect autophagy via a common mechanism.

Since downregulation of Nampt inhibits autophagic flux, we hypothesized that overexpression of Nampt during ischemic stress may protect the heart by stimulating autophagic flux. The size of the myocardial infarct/area at risk after 2 hours of ischemia was significantly smaller in Tg-Nampt than in NTg.[21] Furthermore, the level of autophagic flux was greater in Tg-Nampt than in NTg, as evidenced by less accumulation of p62 in Tg-Nampt.[21] Taken altogether, these results are consistent with the notion that the downregulation of endogenous Nampt observed in the heart during ischemia is detrimental partly due to inhibition of autophagic flux. We speculate that Nampt-induced increases in autophagic flux most likely occur through stimulation of Sirt1 in cardiomyocytes, since Sirt1 has essentially the same effect as Nampt upon autophagy. Furthermore, the close similarity of the *in vivo* phenotypes of Tg-Nampt and Tg-Sirt1 in response to stress [21, 27] suggests that there may be a close functional coupling between Nampt and Sirt1 (sirtuins) in the heart even if they may not have direct physical interaction.

Nampt inhibition by the specific chemical inhibitor FK866 also increases autophagosome formation, as evaluated with GFP-LC3 puncta, in SH-SY5Y neuroblastoma cells. However, since autophagosome accumulation can be observed when autophagic flux is blocked, whether FK866 stimulates autophagic flux remains to be clarified.[6] Recently, Cea et al. showed that Nampt plays an essential role in maintaining both cell viability and intracellular NAD⁺ stores in multiple myeloma cell lines, and that FK866 triggers autophagic, but not apoptotic, cell death.[40] Again, it would be critical to test whether suppression of Nampt by FK866 really stimulates autophagic flux in cancer cells. Nampt is upregulated in cancer cells, which rely on NAD⁺ to support their rapid cell proliferation.[41–43] Interestingly, Nampt promotes survival of neurons, terminally differentiated cells, during cerebral ischemia. This occurs through induction of autophagy via regulation of the TSC2-mTOR-S6K1 pathway in a Sirt1-dependent manner.[44] Thus, Nampt may have distinct effects upon autophagy in terminally differentiated cells and cancer cells.

The effect of Nampt upon cardiac hypertrophy

Pillai et al. showed that agonist-induced cardiac hypertrophy is associated with loss of intracellular levels of NAD⁺. Exogenous addition of NAD⁺ was capable of maintaining intracellular levels of NAD⁺ and blocking the agonist-induced cardiac hypertrophy *in vitro* and *in vivo* through activation of Sirt3.[22] Whether the suppression of hypertrophy is due to the direct effect of NAD⁺ upon the hypertrophy machinery or is secondary to the improvement of cardiac function due to normalization of NAD⁺ content remains to be elucidated. Cai et al. showed that maintenance of the intracellular NAD⁺ level by overexpression of Nmnat2, a form of Nmnat located in the cytoplasm, blocks angiotensin II-induced cardiac hypertrophy in neonatal rat cardiomyocytes.[45] The authors did not

evaluate the tissue level of NAD⁺ in these transgenic mice. However, these results suggest that Nampt is protective against pressure-overload-induced cardiac hypertrophy. Recently, Karamanlidis et al. showed that complex I deficiency caused by genetic deletion of *Ndufs4* causes accumulation of NADH and a decrease in the NAD⁺/NADH ratio, which in turn inhibits Sirt3 and induces hyperacetylation of mitochondrial proteins and mPTP opening. These changes were alleviated by normalization of the NAD⁺/NADH ratio. The complex I deficiency rendered mice highly susceptible to additional stresses and accelerated the development of heart failure after pressure overload or repeated pregnancy. [46] Thus, maintaining the intracellular NAD⁺ level may suppress cardiac hypertrophy and failure. Supplementation of NAD⁺ or enzymes to produce NAD⁺ may be considered for treatment of cardiac hypertrophy and heart failure in the future. Although external application of NAD⁺ has been used to increase the level of NAD⁺ and stimulate NAD⁺-dependent enzymes in the heart or cardiomyocytes, [21,22] the underlying mechanism of uptake and its efficacy are not well understood.

In contrast, using cardiac-specific Nampt overexpression mice, Pillai et al. demonstrated that cardiomyocytes are capable of secreting Nampt during stress and that Nampt is a facilitator of stress-induced cardiac hypertrophy.[14] It should be noted that cardiac hypertrophy has never been observed in the cardiac-specific Nampt overexpression mice we generated.[21] Nampt stimulates NF- κ B, whereas it also inhibits tumor necrosis factor- α (TNF- α)-induced NF- κ B activity.[47] eNampt may protect cells from ER-stress-induced apoptosis by activating an IL-6/STAT3 signaling pathway through a non-enzymatic mechanism.[48] Thus, Nampt appears to have pleiotropic actions and its function may be context-dependent in the heart.[24] Although whether Nampt is secreted from cardiomyocytes remains to be clarified, eNampt may act on non-myocytes, which in turn affect growth and death of cardiomyocytes through paracrine mechanisms. [22]

The effect of Nampt upon cardiac metabolism

Until now, there has been no specific report regarding how Nampt affects cardiac metabolism in response to metabolic perturbation, such as high-fat feeding or nutrient deprivation. Canto et al. showed that dietary supplementation with nicotinamide riboside (NR), a vitamin B3 and NAD⁺ precursor, in mammalian cells and mouse tissues increases NAD⁺ levels and activates Sirt1 and Sirt3, culminating in enhanced oxidative metabolism and protection against high-fat-diet-induced metabolic abnormalities.[49] Khan et al. also found that NR increases mitochondrial biogenesis in skeletal muscle and ameliorates mitochondrial myopathy.[50] Yoshino et al. also demonstrated that nicotinamide mononucleotide (NMN) improves glucose intolerance and lipid profiles in high-fat-diet- and age-induced type 2 diabetic mice, partly through Sirt1 activation.[51] These results show that NAD⁺ and its precursors may have significant effects on metabolism in response to nutrient stress, and that their effects may be attributable to sirtuins in the heart as well. Although Nampt would be expected to exert similar effects on cardiac metabolism, more experiments are needed to prove this hypothesis.

Conclusions

Nampt critically regulates NAD⁺ content, thereby playing an essential role in mediating cell survival by inhibiting apoptosis and stimulating autophagic flux in cardiomyocytes. Preventing downregulation of Nampt inhibits myocardial injury in response to myocardial ischemia and reperfusion as well as other stress conditions in the heart (Table). Although supplementation of NAD⁺ through upregulation of Nampt is generally protective in the heart, the role of Nampt in the heart during stress requires further clarification because eNampt may have cytokine actions that may either positively or negatively affect the survival of cardiomyocytes.

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TableThe cardiac effects of Nampt/NAD⁺ modulation in different models of heart dysfunction

Modulation of Nampt/NAD ⁺	Stress model	Cardiac effects	Reference
Nampt heterozygous (+/-) knockout iNampt overexpression (Transgenic mice)	hypertrophic (isoproterenol and angiotensin II) stimuli Baseline mice without stimulus	Protection against hypertrophy Development cardiac hypertrophy and fibrosis at 6 months of age	[14]
Administration of FK866 (inhibitor of Nampt)	Myocardial ischemia/reperfusion model (<i>in vivo</i> and <i>ex vivo</i>) in mouse	Reduction of myocardial injury only <i>in vivo</i> , but not <i>ex vivo</i> (Reduce neutrophil-mediated injury)	[18]
iNampt overexpression (Transgenic mice)	Myocardial ischemia and ischemia/reperfusion model	Reduction of myocardial injury Stimulation of autophagy	[21]
Exogenous addition of NAD ⁺	hypertrophic (angiotensin II) stimuli	Inhibition of cardiac hypertrophy through activation of Sirt3	[22]
Administration of Nampt (eNampt) at the time of reperfusion	Myocardial ischemia/reperfusion model	Reduction of myocardial injury	[30]
Administration of Nampt (eNampt)	Before H ₂ O ₂ treatment in H9c2 cardiomyocytes	Suppression of myocardial apoptosis	[31]
Overexpression of Nmnat2	hypertrophic (angiotensin II) stimuli	Inhibition of cardiac hypertrophy in neonatal rat cardiomyocytes	[45]
Decrease the NAD ⁺ /NADH ratio by cardiac-specific deletion of <i>Ndufs4</i>	Pressure overload (Transverse aortic constriction) or repeated pregnancy.	Acceleration of heart failure	[46]