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REVIEW ARTICLE

Nicotinamide N-methyltransferase and liver diseases

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Abstract Cellular metabolism-induced epigenetic regulation is essential for the maintenance of cellular homeostasis. Nicotinamide N-methyltransferase (NNMT) is emerging as a key point of intersection between cellular metabolism and epigenetic regulation and has a central role in various physiological and pathological processes. NNMT catalyzes the methylation of nicotinamide (NAM) using the universal methyl donor S-adenosyl methionine (SAM) to yield S-adenosyl-L-homocysteine (SAH) and N1-methylnicotinamide (MNAM), directly linking methylation balance with nicotinamide adenosine dinucleotide (NAD^+) contents. NNMT acts on either the SAM-methylation balance or both NAD^+ metabolism, depending on the tissue involved or pathological settings where metabolic demand is increased. Under physiological conditions, the liver act as an essential metabolic organ with abundant NNMT expression, while NNMT hepatic function is not mediated by its methyltransferase activity due to other major methyltransferases such as glycine N-methyltransferase (GNMT) in the liver. However, hepatic NNMT, as well as its metabolite is improperly regulated and linked to the worse pathological states in liver diseases, including alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), liver cirrhosis, and hepatocellular carcinoma (HCC), suggesting a potential role in the process of liver diseases. In this review, we summarize how NNMT regulates cell methylation balance and NAD metabolism, and extensively outline the current knowledge concerning the functions of NNMT in hepatic metabolism including glucose, lipid and energy, with a specific focus on the contribution of NNMT to the pathophysiology of liver-related diseases. NNMT is involved in the

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development and progression of liver diseases. Understanding the complex NNMT regulatory network and its effects on pathogenesis could provide new therapeutic strategies in the context of liver diseases.

Introduction

Methylation is an essential and ubiquitous reaction that has been shown to be central to various biological processes. It is well known that up to 85% of transmethylation reactions occur in the liver.[1,2] Glycine N-methyltransferase (GNMT), guanidinoacetate N-methyltransferase, and phosphatidylethanolamine N-methyltransferase are the main methyltransferases in the liver, which convert S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH).[3] S-adenosylhomocysteine hydrogenase converts SAH to homocysteine,[4] which is converted back to methionine by methionine synthase using 5-methyl tetrahydrofolate, or by betaine-homocysteine methyltransferase using betaine as a methyl group donor to complete the methionine cycle.[5]

Nicotinamide N-methyltransferase (NNMT), as a key methyltransferase, has been reported that extensively expressed in the liver and adipose tissue.[6] NNMT catalyzes the methylation of nicotinamide (NAM) using the universal methyl donor SAM to yield SAH and N1-methylnicotinamide (MNAM) in the methionine cycle.[6] NAM is metabolized to nicotinamide mononucleotide by nicotinamide phosphoribosyltransferase (NAMPT), then nicotinamide mononucleotide is converted to nicotinamide adenine dinucleotide (NAD^+) in the salvage pathways.[7] Therefore, NNMT links the methionine metabolism and NAD^+. NAD^+ is a central metabolic cofactor for cellular metabolism and energy homeostasis, and NAD^+ metabolism acts as an emerging therapeutic target for several diseases.[8–10] NNMT expression is associated with multiple metabolic parameters in mice and humans suggesting a functionally conserved metabolic role.[11] NNMT can alter post-translational protein modifications and histone methylation by changing global levels of the methyl donor SAM,[12] and is emerging as a key point of intersection between cellular metabolism and epigenetics,[13] as shown in Figure 1.

NNMT has been implicated in the pathogenesis of several diseases including cancer,[14,15] neurodegenerative diseases,[16,17] and metabolic diseases.[18–20] Recent studies have expanded the role of NNMT, it is associated with the regulation of various metabolic pathways in adipose and liver tissues via altering methylation potential (SAM/SAH ratio) and the generation of active metabolites.[11,21,22] The specific role of NNMT in regulating epigenetics and cell metabolism depends on the tissues involved. NNMT may exhibit a beneficial role in the liver but a harmful effect on the adipose tissue.[9] Both NNMT and its metabolite have recently been linked to liver diseases, including alcoholic liver disease,[23] non-alcoholic fatty liver disease (NAFLD),[24] liver cirrhosis, and hepatocellular carcinoma (HCC),[22] suggesting a potential role in the process of liver diseases. In this review, we extensively summarize the emerging findings of NNMT and underlying molecular mechanisms in hepatic metabolism including glucose, lipid and energy, with a specific focus on the contribution of NNMT to the pathophysiology of liver-related diseases.

NAMPT, nicotinamide phosphoribosyltransferase; NMNAT, nicotinamide mononucleotide adenylyltransferase; Sirts, sirtuins; 2PY, N1-methyl-2-pyridone-5-carboxamide; 4PY, N1-methyl-4-pyridone-3-carboxamide.

The role of NNMT in methyl donor balance and epigenetic regulation

It is well known that methyl donor balance, maintained by methionine cycle proteins regenerating SAM, is critical for epigenetic regulation in cells.[24,25] In the methionine cycle, SAM is the main donor in transmethylation reactions and it is synthesized from adenosine triphosphate and methionine. SAM donates the methyl group and becomes SAH.[5] NNMT uses SAM as a methyl donor and NAM as a substrate to produce SAH and MNAM. NNMT acts as a bridge between the methionine cycle and methyl donor metabolism, thereby playing a major role in methyl donor balance.[25]

The liver plays a key role in the disposal of methionine and SAM usage.[26,27] Disruption of the methionine cycle leads to methyl donor imbalance and subsequently causes fatty liver and HCC.[28,29] The liver expresses multiple methyltransferases to maintain the methyl donor balance.[25] It is well known that GNMT is dominantly expressed in the liver, under physiological conditions, which protects the liver from fibrosis and steatosis by avoiding the excessive accumulation of SAM.[30] GNMT decreases in HCC, and prevents the development of HCC by avoiding the hypermethylation of DNA and histones in several key carcinogenesis pathways.[30,31] NNMT is predominately expressed in the liver and adipose tissues. NNMT knockdown increases the SAM content and disturbs methyl donor balance in adipocytes.[19] However, the effect of NNMT on methyl donor balance in the liver is limited by the major methyltransferase GNMT.[32] NNMT alters hepatic methylation potential in GNMT-knockout mice[24] and HCC where GNMT is downregulated or is absent.[32] Deletion of GNMT leads to a hepatic reduction in total transmethylation flux and concomitant accumulation of SAM, which can be compensated by NNMT when exogenous NAM is administrated. GNMT-knockout mice treated with NAM prevents the development of fatty liver and fibrosis by reversing SAM accumulation caused by GNMT deficiency.[24] NNMT is upregulated and contributes to a hepatic methyl donor balance in several liver diseases.[23,33] NNMT overexpression decreases SAM level and the SAM/SAH ratio both in livers and in primary

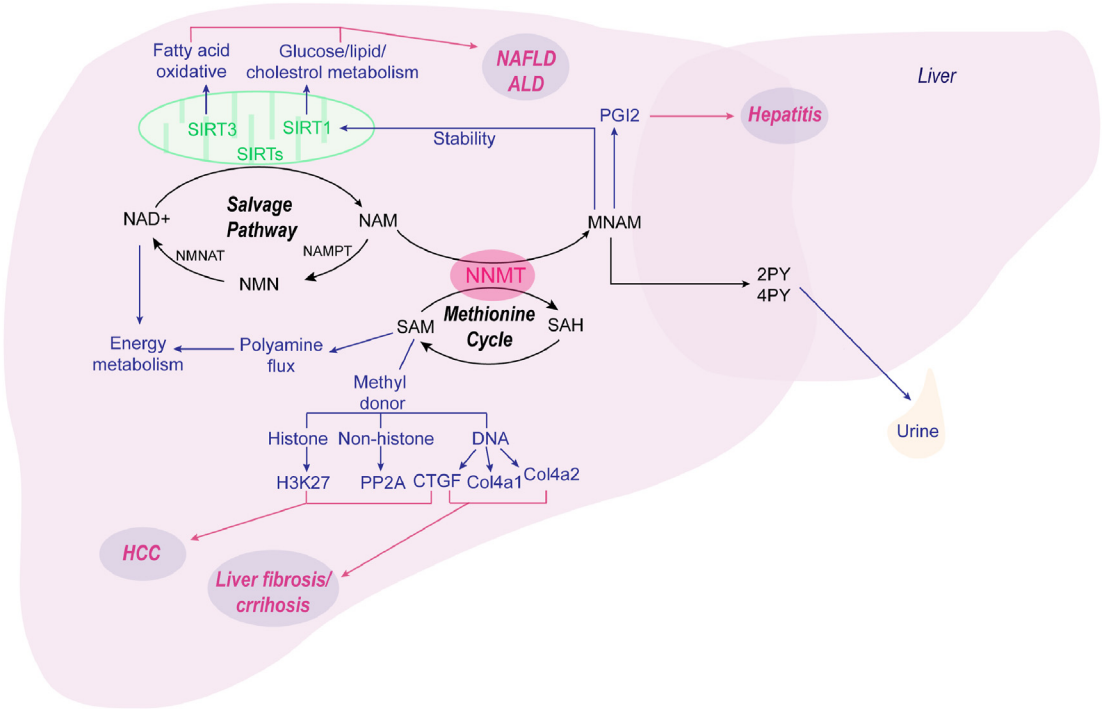

Figure 1 Schematic overview of the roles of NNMT in liver diseases. NNMT consumes the universal methyl donor SAM to methylate NAM, by which reaction NNMT directly links the methionine cycle with the methylation balance and NAD^+ salvage pathway. SAM decreases caused by NNMT activity can change methylation levels of histone, non-histone protein, and DNA, which link to liver diseases, such as HCC, and liver fibrosis/cirrhosis. NNMT regulates the expression and activity of Sirt3 by changing NAD^+ content. Sirt3 is associated with fatty acid oxidative. In addition, MNAM, the product of NNMT, interferes with Sirt1 degradation, leading to deacetylation of Sirt1 targets and suppression of lipid and cholesterol synthesis in hepatocytes. Both Sirt1 and Sirt3, participate in the developing process of NAFLD and ALD. MNAM is also related to hepatitis via the prostacyclin (PGI2)-dependent signaling. MNAM can be further oxidized by Aox into two related compounds, 2PY and 4PY, redundant MNAM and its metabolites are eventually excreted in the urine.

hepatocytes. Conversely, NNMT knockdown has no effect on SAM levels and the SAM/SAH ratio. This limited effect of NNMT knockdown on methyl donor balance results from GNMT dominantly expressed methyltransferase in the liver, as evidenced by NNMT knockdown increases SAM levels and SAM/SAH in hepatocytes with simultaneous GNMT knockdown.[25] Importantly, NNMT, acting as a scaffold, recruits other enzymes in the methionine cycle including betaine-homocysteine methyltransferase, adenosyltransferase 1α, and adenosylhomocysteinase, to aid the recycling of homocysteine to SAM.[25] Collectively, the contribution of NNMT to hepatic methyl donor balance may be dependent on GNMT activity or specific diseases states.

Growing evidence reveals that NNMT can alter the epigenetic state of cells by creating a methyl donor sink and changing methylation potential, especially in cancer cells and adipocytes.[12,19] NNMT has been shown to be overexpressed in a wide range of cancer cells, in which NNMT not only alters methyl donor balance but also promotes hypomethylation of histones as well as other tumor-related proteins.[25] NNMT decreased several histones methylation, such as histone 3 lysine 4 (H3K4), H3K9, H3K27, and H4K20, which were linked to cancer.[12,34] Phosphatase 2A (PP2A), the tumor suppressor, is regulated by methylation,[35] and PP2A methylation deficiency has been illustrated to promote basal extracellular signal-regulated kinase pathway activity which is needed for growth factor responses in cancer cells.[36] PP2A methylation is reduced and demethylation is increased in NNMT overexpression cancer cells, the opposite effects are observed by NNMT inhibition,[12] which is consistent with observations in liver cancer and glioblastoma.[37,38] NNMT may alter histone methylation in a methylation-site-specific manner rather than a global manner. Emerging evidence showing this selective effect of NNMT might depend on the relative Km and Ki values of individual methyltransferase enzymes, including NNMT itself, for SAM and SAH, respectively.[12] Take H3K27 and PP2A methylation as examples, PP2A is methylated by leucine carboxyl methyltransferase 1 and H3K27 is methylated by enhancer of zeste homolog 1 (EZH1) and EZH2.[39,40] All of these methyltransferases have higher Km values for SAM and SAH, which causes PP2A and H3K27 more sensitive to NNMT compared with other methyltransferases that have lower Km values for SAM, such as the histone coactivator-associated arginine methyltransferase 1 (H3R17me2a).[12,13] Further study is warranted to elucidate how NNMT regulates specific gene expression changes by altering site-specific methylation events.

The role of NNMT in hepatic metabolism

Glucose metabolism

The coordinating role of insulin and glucagon guarantees that glucose homeostasis is kept in a wide range of physiological conditions.[41] The liver is one of the vital target organs for insulin and is the source of 90% of endogenic glucose production.[42]

It has been shown that NNMT regulates glucose metabolism.[21] In primary hepatocytes, NNMT knockdown significantly decreases hepatocyte glucose production and inhibits glucose-6-phosphatase catalytic (G6pc) and phosphoenolpyruvate carboxykinase 1 cytosolic (Pck1) expression. In contrast, NNMT overexpression shows higher glucose output, and higher G6pc and Pck1 gene expression compared with control hepatocytes.[21] This finding is confirmed in mice with NNMT knockdown. NNMT-knockdown mice have lower fasting glucose levels, lower pyruvate conversion to glucose, and lower G6pc and fructose bisphosphatase 1 gene expression.[21] These data indicate that NNMT may act as a positive regulator of hepatic glucose metabolism.

Sirtuin 1 (Sirt1) is a critical regulator of gluconeogenesis. Sirt1, as a deacetylation protein, can regulate gluconeogenesis through deacetylation of the peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), forkhead box O 1 (FOXO1), and other factors.[43–45] Sirt1 protein, but not mRNA can be altered by NNMT expression, suggesting NNMT might regulate Sirt1 protein stability.[21] Indeed, NNMT can regulate the ubiquitin-proteasome degradation of Sirt1, thereby stabilizing Sirt1 protein.[21] In line with NNMT overexpression, MNAM, the product of NNMT, also increases glucose production, G6pc and Pck1 expression in hepatocytes, while these alterations are abolished by Sirt1 knockdown.[21] MNAM blocks the changes in fasting glucose and insulin induced by a high-fat diet (HFD) via Sirt1.[21] This finding is consistent with a recent study showing that MNAM can activate Sirt1 while decrease forkhead box O 1 acetylation and down-regulate several key mediators in the gluconeogenesis, such as Pck1 and G6Pc, consequently leading to a reduction of hepatic glucose production and improvement of insulin resistance in obese type 2 diabetes mellitus mice.[46]

In contrast to the metabolic effects on the liver, white adipose tissue NNMT expression and plasma MNAM correlates positively with insulin resistance,[47] and antisense oligonucleotide knockdown of NNMT in adipose tissue and liver decreases serum insulin levels and glucose-insulin product, and improves glucose tolerance.[19] The underlying mechanism of this action offered by NNMT in the liver is different from that in adipose tissue. In the liver, the SAM and NAD^+ levels are not changed by NNMT, and the metabolic actions of NNMT are mediated by MNAM via stabilizing Sirt1 protein.[21] In adipose tissue, NNMT regulates SAM and NAD^+ levels, which impact histone methylation, polyamine flux and Sirt1 signaling.[19] NNMT directly regulates SAM in adipose tissue, but not in the liver, due to NNMT is not a major methyltransferase, and hepatic SAM is regulated by other methyltransferases which contribute to the methyl donor flux.[24] Adipose NAD^+ synthesis is primarily controlled by a salvage pathway using NAM and NNMT is the only catabolic enzyme for NAM. Liver NAD^+ synthesis relies on a salvage pathway using NAM and a *de novo* pathway using tryptophan.[48]

Lipid metabolism

Hepatic NNMT is significantly upregulated in HFD-fed mice either in the acute phase or chronic phase, and correlates with the development of obesity and diabetes.[19,47,49] Overexpression of NNMT in the liver may be associated with the pathogenesis of non-alcoholic- and alcohol-related fatty liver disease. Inhibiting NNMT expression protects mice from diet-induced obesity and decreases serum triglycerides and free fatty acids.[19] Transgenic NNMT overexpression mice fed an HFD supplemented with NAM, a precursor for NAD^+, exhibiting fatty liver deterioration and a reduction in very-low-density lipoprotein (VLDL) secretion.[33] The expression of the genes related to lipid uptake, such as the cluster of differentiation (CD) 36 and lipoprotein lipase are increased, while hepatic lipogenesis-related genes, such as sterol regulatory element-binding protein 1c (SREBP1c), are decreased.[33] It has become increasingly evident that Sirt3 can regulate fatty acid oxidation and oxidative stress.[50,51] Indeed, Sirt3 activity, but not Sirt1 activity decreases via reducing NAD^+ content in the NNMT transgene mice with NAM.[33] NNMT overactivation also decreases genes related to fatty acid oxidation, such as acyl-coenzyme A oxidase (Acox) 1, medium-chain acyl-CoA dehydrogenase, and long-chain acyl-CoA dehydrogenase by inhibiting NAD^+-dependent deacetylase Sirt3 function.[33] In line with this finding, adenoviral NNMT knockdown, as well as NNMT inhibitor administration prevents fatty liver development in response to chronic alcohol exposure via decreasing intracellular triacylglycerol concentration, and down-regulating a range of genes associated with *de novo* lipogenesis pathway, such as sterol regulatory element binding transcription factor1 (Srebf1), acetyl-CoA carboxylase alpha (Acaca) and acetyl-CoA carboxylase beta (Acacb).[23] However, NNMT knockdown does affect adipose tissue lipolysis.[23] Importantly, the inhibitory of the lipogenic pathway by NNMT inhibition appears to not be related to its NAD^+-enhancing action, but links with the activation of serine/threonine kinase AMP-activated protein kinase.[23] Due to overexpression of NNMT reduces intracellular methylation status potentially resulting from depleted SAM,[33] and the reduced SAM content is linked with elevated SREBP1-dependent *de novo* lipogenesis,[52] it is possible that SAM-regulated methylation reactions also involves this process.[23]

In contrast, hepatic expression of NNMT is inversely associated with multiple metabolic parameters such as serum high-density lipoprotein, total cholesterol, and triglyceride levels both in mice and humans.[21] NNMT knockdown mice alter glucose and cholesterol metabolism, whereas supplementation of MNAM, the product of NNMT, improves the metabolic profile of HFD-fed mice. The metabolic effects of NNMT in the liver are related to its ability to stabilize Sirt1 protein.[21] Sirt1 upregulates gluconeogenesis, whereas suppresses cholesterol synthesis and lipogenesis,[53] and thus, by increasing Sirt1 protein stability,

NNMT mediates the regulation of cholesterol synthesis and lipogenesis.[21] Therefore, the activity of NNMT as a stabilizer of Sirt1 might enable the liver to cope with the accumulation of body fat caused by overnutrition.[21] Further researches are needed to delineate and clarify the role of NNMT on lipid metabolism and precise underlying molecular mechanisms.

Energy metabolism

NAD^+ as well as SAM, are fundamental metabolites for energy metabolism. NAD^+ is a critical coenzyme for redox reactions and interconversion of different classes of metabolites, including the conversion of glucose to lipids, making it is central to energy metabolism.[10] NAD^+ is also an important cofactor for non-redox NAD^+-dependent enzymes, including sirtuins and poly(ADP-ribose) polymerases, affecting a large array of cellular functions.[10] Histone methyltransferases (HMTs) use SAM as a coenzyme to transfer methyl groups to lysine and arginine residues of histone proteins. Histone methylation plays a critical role in transcriptional regulation.[54] Polyamines, organic polycations, are largely bound to RNA and DNA and essential for many cellular functions affecting transcription and cell growth.[54] SAM regulates energy metabolism by providing methyl groups for histones, and propylamine groups for polyamine metabolism. NNMT is the major NAM-metabolizing enzyme that catalyzes the methionine cycle and also regulates the biosynthesis of NAD^+ by metabolizing NAM. Thus, NNMT couples NAD^+ metabolism with the methionine cycle.[11]

It is conceivable to postulate that NNMT influences NAD^+ metabolism in the liver. Indeed, NNMT knockdown increases in hepatic NAD^+ levels in mice exposed to chronic alcohol consumption, whereas NNMT overexpression in AML12 hepatocytes reduces intracellular NAD^+.[23] In transgenic NNMT overexpression mice, hepatic SAM/SAH ratio and NAD^+ levels are significantly lower than those in wide type mice when both fed with NAM-supplemented HFD, while hepatic NAD^+ metabolite is not significantly different between the transgenic mice and wild type mice under HFD without NAM.[33] NNMT knockdown also does not affect either the SAM/SAH ratio or NAD^+ content in primary hepatocytes and the livers of mice.[21] Due to NNMT is not the main methyltransferase in the liver,[11] it is possible that NNMT is not enough to change the hepatic balance between NAD^+ metabolism and methyl donor under HFD. Thus, only under NAM-replete conditions, such as under NAM-rich food intake, NNMT can play a crucial role in the NAD^+ metabolism and methionine cycle.[55]

Besides the modulation of NAD^+ content and alteration of methionine cycle to change methylation potential, NNMT might influence polyamine flux.[18] SAM is a substrate for polyamine metabolism. SAM decarboxylase can decarboxylate SAM to decarboxy-SAM and enter the polyamine cycle.[54] Polyamine flux is regulated by rate-limiting enzymes such as ornithine decarboxylase (ODC), spermidine-spermine N1-acetyltransferase (SSAT), and polyamine oxidase (PAO), which play essential roles in energy homeostasis.[56] SSAT knockout leads to increased diet-induced obesity, in contrast, transgenic SSAT overexpression improves metabolic profile, which owing to enhanced energy expenditure via accelerating polyamine flux.[56,57] NNMT inhibition increases adipose ODC and SSAT activity and urinary diacetylspermine excretion, convincingly suggesting that NNMT directly regulates polyamine flux in adipocytes.[19] Mechanically, NNMT inhibition increases ODC and SSAT expression by modifying H3K4me0, thereby causing polyamine flux activation. NNMT inhibition autonomously enhanced-oxygen consumption is abolished by inhibiting ODC, SSAT, or PAO activity, respectively, indicating it depends on polyamine flux.[19]

The role of NNMT in liver diseases

Alcoholic liver disease (ALD)

ALD, a disorder caused by excessive alcohol intake, characterizes as a global healthcare burden. The pathological process of disease development includes asymptomatic steatosis, alcoholic steatohepatitis, with some individuals ultimately progressing to fibrosis, cirrhosis, and HCC.[58,59]

Chronic alcohol consumption activates endoplasmic reticulum (ER) stress and upregulates NNMT expression by activating the PRKR-like ER kinase-transcription factor 4 (PERK-ATF4) pathway in the liver.[23] The upregulated NNMT expression is associated with the incremental its product MNAM content, indicating increased NNMT enzymatic activity.[23] Importantly, inhibiting NNMT activity either by NNMT knockdown or chemical inhibitors protects against alcohol-related fatty liver development by suppressing the *de novo* lipogenesis genes including Srebf1, Acaca, Acacb, and fatty acid synthase, which is NAD^+-independent.[23] Moreover, ALD mice fed supplementation with exogenic SAM, NAM, or nicotinic acid protects mice against ALD, implying that increased hepatic MNAM derived from NNMT activation may contribute to hepatic fat deposition.[23] To complement these findings, Ding et al demonstrated that MNAM supplementation results in hepatic lipid accumulation in alcohol-fed mice, and aggravates oleic acid-induced hepatic triacylglycerol accumulation in AML-12 hepatocytes.[60] In contrast to its adverse effect on hepatic steatosis, MNAM supplementation alleviates liver injury in response to chronic alcohol exposure.[60] Similarly, transgenic NNMT overexpression aggravates hepatic steatosis but reduces liver injury by chronic alcohol feeding, suggesting that NNMT upregulation can differentially regulate liver fat accumulation and hepatotoxicity in response to chronic alcohol feeding.[60] However, Song and colleagues showed that hepatic steatosis, but not liver damage is observed in the presence of chronic alcohol exposure for 5 weeks.[23] Although several studies have been revealed the protective effects of MNAM in various liver injury models,[61] the alleviation effect of exogenous MNAM in ALD has been challenged.[62] Because MNAM is not only a production of NNMT but also an allosteric inhibitor of it,[6] the amelioration role of exogenous MNAM on ALD-induced liver injury might result from NNMT inhibition.[62] Indeed, NNMT inhibition, by using highly specific NNMT inhibitors, JBSNF-000088 and II399, prevents palmitate-induced intracellular triacylglycerol accumulation and cell death in AML-12 hepatocytes.[62]

Collectively, NNMT may act as a risk factor for ALD, and targeting NNMT might be a potential treatment for ALD.

Non-alcoholic fatty liver disease (NAFLD)

NAFLD involves two entities named non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFL is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury either by imaging or by histology in individuals without significant alcohol consumption and secondary causes of steatosis are absent. NASH is characterized by the presence of both steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis.[63,64]

Unlike NNMT is a risk factor in ALD, NNMT may be a possible protective factor for NAFLD.[21,65] Overexpressing hepatic NNMT or maintaining MNAM concentrations could improve lipid parameters and ameliorate fatty liver.[21,65] Acox2, encoding a branched-chain acyl-CoA oxidase involved in the degradation of long branched fatty acids and bile acid intermediates in peroxisomes, can act as a candidate causative gene for NAFLD.[66,67] In the Acox2 deficiency mice, NAFLD phenotype, including steatosis, inflammation, and fibrosis appears at the age of 4 months.[20] Moreover, NNMT expression, as well as its product MNAM is reduced in Acox2 loss-induced NAFLD, suggesting that decreases in NNMT and that of MNAM are risks for NAFLD.[20] However, whether MNAM reduction results from the down-regulation of NNMT or overactivation of its degradation, and whether MNAM reduction is a contributor to the development and progression in Acox2 loss-induced NAFLD are still under investigation.

On the contrary, NNMT is upregulated and related to fatty liver development in HFD-fed mice.[19,33] NNMT knockdown protects against HFD-induced obesity by augmenting cellular energy expenditure via regulating histone methylation, polyamine flux, and Sirt1 signaling.[19] Transgenic NNMT overexpression mice fed an HFD containing NAM, showed that body weight and fat mass are reduced while hepatomegaly, accumulation of hepatic triglyceride content, NAFLD activity score, and fibrosis factors were increased. These beneficial effects of NNMT were mediated by increased expression of genes related to lipid uptake and decreased VLDL secretion.[33] NNMT overactivation also reduces the expression of genes related to fatty acid oxidation by inhibiting NAD^+-dependent deacetylase Sirt3 function.[33] Sirt3, one of a family of histone deacetylases, locates in mitochondria and regulates mitochondrial function involved in fatty acid oxidation and oxidative stress, and plays a vital role in steatohepatitis.[50,51] Both Sirt3 expression and activity have been shown to significantly be reduced under the NNMT overactivation condition, which is mediated by NAD^+ reduction. Sirt3-deficient mice are susceptible to NASH induced by a methionine-choline-deficient diet by increasing oxidative stress, liver inflammation, and fibrosis.[68] Additionally, hepatic lipotoxicity plays a pathological role in NAFLD. The cellular and molecular mechanisms underlying lipotoxicity remain to be elucidated. Recently investigators have examined the effects of NNMT on palmitate-induced lipotoxicity in hepatocytes.[69] They demonstrate that NNMT upregulation contributes to palmitate-induced hepatotoxicity as NNMT inhibition, via either pharmacological (NNMT inhibitors) or genetic approach (siRNA transfection), protected palmitate lipotoxicity. Moreover, a recent study shows that an NNMT inhibitor, 5-amino-1-methylquinolinium, could improve liver steatosis in western diet-induced obese mice via producing a unique metabolomic signature in adipose tissue.[70] As the etiology for NAFLD is very heterogeneous, and the diet-induced model as well as observation periods are different, it is possible to explain this contradictory effect of NNMT or its inhibitors on NAFLD.

Liver fibrosis and cirrhosis

Liver cirrhosis is the final pathological change of liver injury arising from multiple chronic liver diseases.[71,72] Methylation reactions in the liver are significantly influenced by the presence of liver diseases including liver cirrhosis. Cuomo et al investigated the methylation of NAM in patients with cirrhosis, and demonstrated that the basal serum levels of MNAM were significantly higher in patients with cirrhosis, and serum levels of MNAM were also higher after the NAM oral load.[1] In line with this finding, Pumpo and colleagues showed that serum and urinary levels of MNAM, as well as its metabolite, 2-pyridone-5-carboxamide (2-PY) in urine are increased in cirrhotic patients in basal conditions and after a NAM oral load, while the ratio of MNAM/2-PY was similar, suggesting that the increased NAM methylation may be against the toxic effect of the intracellular accumulation of NAM deriving from the catabolic state of cirrhosis.[73]

Liver fibrosis, the precursor of cirrhosis, is characterized by the excessive accumulation of extracellular matrix proteins with collagen.[74,75] A recent study demonstrates that HFD with NAM induces liver fibrosis, and the genes involved in extracellular matrix regulation, such as collagen (COL) 1, COL4A1, COL4A2, and connective tissue growth factor (CTGF) are increased in NNMT transgenic mice.[33] CTGF has been confirmed as the main contributor to liver fibrosis,[76] and the CTGF promoter is hypomethylated in NNMT transgenic mice after administration of a NAM-supplemented HFD.[25] Due to CTGF gene expression being mediated by its methylation status of the CpG island,[77] therefore, demethylation of CTGF by NNMT overexpression may contribute to the pathogenesis of liver fibrosis. Inhibition of NNMT did not lead to liver fibrosis as indicated by normal expression hepatic fibrosis markers metal-loproteinase 1 and COL1 in mice fed with HFD.[19] Collectively, these findings suggested that NNMT activation can drive the depletion of both NAD^+ and SAM when NAM was abundantly available, thereby resulting in liver steatosis and fibrosis.[25] Given that NAM intake and NNMT activation are increased in obesity,[19,78] targeting NNMT may provide a potential novel strategy for treating fatty liver and liver fibrosis.

Hepatitis

Concanavalin A (Con A)-induced hepatitis is a widely used acute immune-mediated hepatitis model, which is primarily motivated by the stimulation and recruitment of T cells to the liver compared to other hepatic injury models.[79,80]

NNMT activity in the liver is significantly upregulated 8—24 h after Con A injection, meanwhile, its metabolites 2-PY and 4-pyridone-5-carboxamide are increased in plasma.[61] Exogenous MNAM administration prevents ConA-induced hepatitis, and the protective effect is lost in the presence of an antagonist of the prostacyclin (PGI2) receptor, suggesting that MNAM might limit liver injury by a PGI2-dependent mechanism. They further confirm the concept that the protective effect on the Con A-induced liver injury is PGI2-dependent involving down-regulation of interleukin-4 release from lymphocytes and tumor necrosis factor-alpha (TNFα) release from Kupffer cells.[81] In addition, Taniki et al found that NNMT is upregulated in a murine tandem model of dextran sulfate sodium-Con A hepatitis.[82] Apart from Con A-induced hepatitis, hepatic NNMT is also increased in fulminant hepatic failure mice.[83,84] Moreover, NNMT is increased in the human hepatoma-derived cell line when treated with hepatitis C virus core protein.[85] These results collectively suggest that NNMT is increased and plays a vital role in the progression of liver hepatitis. However, the role of NNMT and precise molecular mechanisms underlying this process remain to be characterized.

Liver cancer

It has been observed that NNMT upregulates in a variety of cancer cells, such as breast cancer,[86] glioblastoma,[87] renal clear cell carcinoma,[88] and bladder cancer.[89] HCC is the end-stage of various chronic liver diseases. Chronic liver diseases are characterized by hepatitis, fibrosis, aberrant hepatocyte regeneration and then even cirrhosis, accompanied by molecular alterations, such as proliferative, invasive, survival advantages, and the transition to HCC.[90] It is well known that the development of HCC is derived from various aberrant methylation processes in the liver.[91] Kim et al showed that NNMT is significantly downregulated in HCC tissues compared to normal adjacent tissues, however, within the HCC tissues, higher NNMT expression is significantly correlated with tumor stage, and NNMT expression is higher in recurrent tumors than in non-recurrent tumors.[22] Consistently, Li et al demonstrated that the expression levels of NNMT in HCC tissues are higher than those in normal adjacent tissues, and also are positively correlated with poor prognosis.[32]

Nevertheless, the role of NNMT in the HCC remains unclear. Hepatic stellate cells (HSCs) are the major cells associated with the progression of various liver diseases, such as hepatitis, fibrosis, and cirrhosis to liver cancer. The intercellular communication between HSCs and HCC cells results in tumor growth, metastasis, and angiogenesis.[92—94] Li et al recently found that activated HSCs increase NNMT levels in HCC cells, thereby increasing HCC invasion and metastasis by activating clusters of differentiation 44 (CD44) via modulating H3K27 methylation.[32] CD44 is important for tumor cell metastasis and progression, and higher CD44 levels are strongly related to tumor metastasis, invasion, and prognosis.[95] CD44v3 variant is abundantly expressed on tumor cells and closely associated with tumor metastasis.[96] NNMT facilitated CD44v3 variant formation via impairing CD44 mRNA m6A modification, while also preventing ubiquitin-mediated CD44 protein degradation by its product MNAM.[32] Furthermore, the effects of NNMT on HCC metastasis may also be related to CTGF. It has been demonstrated that CTGF is overexpressed in HCC tissues compared to normal adjacent tissues, and increased CTGF expression is associated with clinicopathologic malignancy of HCC.[97] Furthermore, hepatocyte-specific knockout of CTGF decreases HCC progression, and CTGF derives from tumor cells acting as a keystone activating nearby HSC.[97] NNMT overexpression upregulates CTGF mRNA level by altering methylation potential.[33] Therefore, it is possible that NNMT also confers an invasive phenotype on HCC cells via modulating CTGF. Shin et al recently revealed that NNMT depletion contributes to liver cancer cell survival and tumor growth by enhancing autophagy via increasing PP2A methylation and subsequent phosphatase activity, providing new insights into the mechanisms of NNMT on liver cancers.[40]

Hepatoblastoma (HBL) is the most common primary malignant hepatic tumor in children.[98] NNMT methylation detected in the fetal liver is lost in the differentiated liver and with inverse correlation on gene expression.[99] However, a recent study finds the promoter region of NNMT is hypermethylated in HBL. NNMT expression is significantly decreased in HBL, and lower NNMT expression is observed in tumor samples and HBL cell lines than those in HCC cell lines.[100] Hypermethylation of a specific TSS1500 CpG site (cg02094283) of NNMT is observed in HBL, and hypermethylation is inversely correlated with NNMT expression.[100] In addition, NNMT might be a potential predictive biomarker for HBL as evidenced by higher NNMT is linked to the late hepatoblastoma diagnosis.[100] Cancer cells have the ability to use lipids as an alternative source of energy for tumor progression.[101—103] Lipids are reduced in HBL and exhibit a negative correlation with NNMT expression, suggesting that NNMT downregulation might diminish the lipid content in HBL.[100] This hypothesis is supported by a study showing NNMT downregulation decreases the lipid content in liver cells.[33] Taken together, NNMT might promote HCC metastasis, and targeting NNMT may present as a potential treatment for liver cancer.

Conclusion

NNMT is a one-carbon group methyltransferase that catalyzes NAM using the universal donor SAM, directly linking cell methylation balance and NAD^+ metabolism. SAM, as well as NAD is a central energy metabolite. Cells sense the methylation balance via modulating SAM and SAH levels, which in turn modifies enzymes, such as HMTs and DNA methyltransferases, to modulate histone methylation levels and ultimately regulate gene expression. NAM is a precursor of NAD^+ which has a key role in modulating energy metabolism including glycolysis, the tricarboxylic acid cycle, and fatty acid oxidation and alcohol (ethanol) metabolism, and is a co-substrate for various enzymes, such as sirtuins.[8,104] NAD^+ can also act as a nucleotide analog in DNA ligation and RNA capping.[105,106] Given that SAM is the universal methyl donor for proteins, nucleotide acids and lipids methylation, NNMT overexpression impairs the genome methylation by

creating a metabolic methylation sink.[12,15] Moreover, NNMT diverts the NAM into producing MMNA, making NAM unavailable for NAD^+ salvage pathway that reduces NAD^+, consequently limiting NAD^+ dependent processes via post-synthesis modification of fundamental biomolecules.[10]

NNMT acts on either the SAM-methylation balance or both NAD metabolism, depending on the tissue involved or pathological settings where metabolic demand is increased. The liver and adipose tissue are the major metabolic organs with abundant NNMT expression. In the adipose tissue, NNMT is the major methyltransferase and regulates methylation potential and nicotinamide degradation, thereby serving as a bad actor in fat.[9] Under physiological conditions, hepatic function of NNMT is not mediated by a methyltransferase activity. The methylation balance in the liver is controlled by other major methyltransferases such as GNMT. Under liver diseases, NNMT may regulate the methionine cycle via competing for SAM with other major methyltransferase reactions, leading to reduced hepatic NAD^+ and SAM levels.[24] The contradictory physiological and pathological role in the liver encourages further research to better understand the molecular patterns involved in NNMT regulatory networks. Furthermore, NNMT may aid in fat storage in the adipose tissue, while may function as a Sirt1 stabilizer to consume the accumulation of body fat produced by episodes of overnutrition.[33] Liver-adipose tissue crosstalk functions as the key mediator in the pathogenesis of metabolic disease. Whether and how the liver can crosstalk with adipose tissue via NNMT to regulate lipid and glucose metabolism remain unknown.

Although, exciting and emerging strides in the understanding of the pathogenesis of liver diseases and NNMT biology in recent years, further investigations are still required to fully exploit the potential role of NNMT in the initiation and progression of liver diseases. NNMT expression may be regulated in a complex and tissue- and context-specific manner. NNMT is upregulated in steatosis, fibrosis and cirrhosis, whereas is decreased in HCC. It is possible that the haptic NNMT function impairs during the progression to HCC and restores in the late stage to precede tumor invasion and metastasis.[32] ER stress, specifically PERK-ATF4 pathway activation, modulates the upregulation of hepatic NNMT in response to chronic alcohol exposure.[23] Whether and how liver disorders influence hepatic NNMT expression, as well as whether and how altered hepatic NNMT contributes to its development remains unclear.

Despite several NNMT inhibits have been recently developed and treated various diseases, including metabolic and liver diseases, multiple cancers and aging,[105–107] the direct outcome of NNMT has been hindered because of their low selectivity and metabolic stability and/or poor cellular permeability.[107,108] Future studies are needed to develop high selectivity and permeability of NNMT inhibitors for deciphering the function and underlying mechanisms of NNMT and ultimately to assess their therapeutic potential.

Conflict of interests

The authors declare that there are no competing interests associated with the manuscript.

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