Methionine adenosyltransferases in cancers: Mechanisms of dysregulation and implications for therapy

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Impact statement

This review examines the role of methionine adenosyltransferases (MATs) in human cancer development, with a particular focus on liver cancers in which all three MAT genes are implicated in tumorigenesis. An overview of MAT genes, isoenzymes and their regulation provide context for understanding consequences of dysregulation. Highlighting examples from liver, colon, gastric, breast, pancreas and prostate cancers underscore the importance of understanding MAT's tumorigenic role in identifying future targets for cancer therapy.

Abstract

Methionine adenosyltransferase genes encode enzymes responsible for the biosynthesis of S-adenosylmethionine, the principal biological methyl donor and precursor of polyamines and glutathione. Mammalian cells express three genes – *MAT1A*, *MAT2A*, and *MAT2B* – with distinct expression and functions. *MAT1A* is mainly expressed in the liver and maintains the differentiated states of both hepatocytes and bile duct epithelial cells. Conversely, *MAT2A* and *MAT2B* are widely distributed in non-parenchymal cells of the liver and extrahepatic tissues. Increasing evidence suggests that methionine adenosyltransferases play significant roles in the development of cancers. Liver cancers, namely hepatocellular carcinoma and cholangiocarcinoma, involve dysregulation of all three methionine adenosyltransferase genes. MAT1A reduction is associated with increased oxidative stress, progenitor cell expansion, genomic instability, and other mechanisms implicated in tumorigenesis. MAT2A/MAT2B induction confers growth and survival advantage to cancerous cells, enhancing tumor migration. Highlighted examples from colon, gastric, breast, pancreas and prostate cancer studies further underscore methionine adenosyltransferase genes' role beyond the liver in cancer development. In this subset of extra-hepatic cancers, MAT2A and MAT2B are induced via different regulatory mechanisms. Understanding the role of methionine adenosyltransferase genes in tumorigenesis helps identify attributes of these genes that may serve as valuable targets for therapy. While S-adenosylmethionine, and its metabolite, methylthioadenosine, have been largely explored as therapeutic interventions, targets aimed at regulation of MAT gene expression and methionine adenosyltransferase protein–protein interactions are now surfacing as potential effective strategies for treatment and chemoprevention of cancers.

Keywords: Chemoprevention, cholangiocarcinoma, colon cancer, hepatocellular carcinoma, methionine adenosyltransferase, S-adenoylmethionine

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MAT genes and isoenzymes

MATs are an essential enzyme family that synthesize S-adenosylmethionine (SAMe), the main methyl donor and precursor in polyamine and glutathione synthesis.[1] SAMe is vital to the methylation of nucleic acids, phospholipids, histones, biologic amines, and proteins; thus, changes in its biosynthesis can profoundly affect cellular growth, differentiation, and function. Recent data also suggest that MATs play significant roles apart from SAMe synthesis, most notably, serving as transcription factors and co-factors, as well as

part of a scaffold complex implicated in cancer development (see below).

Mammalian cells express three genes – *MAT1A*, *MAT2A*, and *MAT2B* – with distinct expression and functions. *MAT1A* is mainly expressed in the liver and maintains the differentiated states of both hepatocytes and bile duct epithelial cells.[1,2] *MAT1A* encodes the catalytic subunit α1, which forms a homo-dimer (MATIII) and tetramer (MATI).[3] Conversely, *MAT2A* and *MAT2B* are widely distributed in non-parenchymal cells of the liver and extrahepatic tissues. *MAT2A* encodes the catalytic α2 subunit found in MATII, predominates in fetal liver and is replaced by *MAT1A* shortly after birth.[4,5] *MAT2B* encodes the regulatory subunit MATβ, which regulates MATII activity by lowering its K_m for methionine and its K_i for SAMe.[6,7] *MAT2B* additionally encodes two major splicing variants, V1 (same as MATβ) and V2, which differs from V1 in the first 20 amino acids at the N-terminus.[8] Both MAT2B variants regulate important processes apart from MATII activity, as they interact with many proteins that affect cell growth and signaling pathways (see below). MATI and MATIII have higher K_ms than MATII and are not feedback inhibited by SAMe. These differences in kinetic and regulatory properties between the MAT isoenzymes allow a marked increase in hepatic SAMe level after a high methionine load.[1] This is important, as the liver is the main site of methionine catabolism, where half of daily intake is converted to SAMe.[1] Table 1 summarizes the three *MAT* genes, isoenzymes, kinetic and regulatory properties of the isoenzymes.

MAT gene regulation and dysregulation

All three MAT genes are regulated at transcriptional, post-transcriptional and post-translational levels. This section describes these regulatory mechanisms for each of the human MAT genes in the normal setting and in cancers. The following section describes the consequences of their dysregulation.

MAT1A

Epigenetic mechanisms and transcription factors are involved in *MAT1A* transcriptional regulation. In normal liver, *MAT1A* is epigenetically upregulated by hyperacetylation and cytosine hypomethylation upstream of the *MAT1A* transcription start site.[9] Hypermethylation of the *MAT1A* promoter is observed in hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA).[2,9,10] Methylation of the coding region also reduces *MAT1A* transcription.[11] Importantly, hypermethylation of the *MAT1A* promoter and coding region was reported in patients with advanced non-alcoholic fatty liver disease (NAFLD) with fibrosis score 3–4, but not mild NAFLD.[12]

The *MAT1A* promoter contains consensus binding sites for multiple transcription factors/co-factors that include: glucocorticoid, interleukin-6 (IL-6), hepatocyte nuclear factor (HNF), activator protein 1 (AP-1), CCAAT enhancer binding protein (C/EBP), cyclic AMP response element binding protein (CREBP), E2F, signal transducers and activators of transcription (STAT), c-MYC and v-MYB.[13] Among these transcription factors, glucocorticoid and C/EBP demonstrate positive regulation of human *MAT1A* transcription.[13,14] Recently, c-MYC, MAF BZIP transcription factor G (MAFG), and c-MAF were shown to negatively regulation the human *MAT1A* promoter.[2] All three proteins are upregulated in both HCC and CCA, and repress *MAT1A* transcription by interacting at the *MAT1A* promoter's E-box region.[2]

Multiple factors downregulate *MAT1A* post-transcriptionally. Binding of AU-rich RNA binding factor (AUF1) to its 3′- untranslated region negatively regulates *MAT1A* mRNA.[15] AUF1 expression is high in HCC and fetal liver and falls during liver development, coinciding with increased *MAT1A* expression.[15] Furthermore, *MAT1A* mRNA level is downregulated by microRNAs (miRs) in HCC.[16,17] Preneoplastic liver lesions induced by 2-acetylaminofluorene injections in rats caused induction of miR-22 and miR-29b that inhibited *Mat1a* mRNA expression.[16] MicroRNAs miR-485–3p, miR-495 and miR-664 are induced in human HCC and all three negatively regulate

Table 1. Mammalian MAT genes and isoenzymes.

MAT gene	Protein product	MAT isoenzyme	Regulatory subunit	K_m for methionine	K_i for SAMe
MAT1A	α1	MATI (tetramer) MATIII (dimer)	None	MATI: 23 μM–1 mM MATIII: 210 μM –7 mM	MATI: 400 μM MATIII: none
MAT2A	α2	Catalytic subunit of MATII	Yes, both V1 and V2 regulate MATII by lowering the K_m for methionine, V1 also lowers the K_i for SAMe (V2 has not been examined)	4–10 μM	60 μM
MAT2B	V1 (same as β), V2	Regulatory subunit of MATII			

MAT: methionine adenosyltransferase.

MAT1A mRNA directly.[17] Reduced MAT1A expression resulted in a lower nuclear SAMe level, hypomethylation of the *LIN28B* promoter region and increased LIN28B expression.[17] Importantly, *LIN28B* is an oncogene that exerts reciprocal regulation with Let-7, a tumor suppressor. Blocking expression of these miRNAs recovers *MAT1A* expression, functionally inhibits growth, induces apoptosis in HCC cell lines, and inhibits HCC growth *in vivo*.[17]

Post-translationally, MATI/III is inactivated by covalent modification of cysteine 121 either by oxidation or nitrosylation.[1,18] This is observed in multiple liver diseases and explains the fall in hepatic MAT activity. High GSH level can reverse cysteine 121 covalent modification; however, GSH is often reduced in chronic liver disease leading to a vicious feed-forward cycle. MATI/III can be phosphorylated by protein kinase C,[19] but this does not alter enzymatic activity.

MAT2A

MAT2A is expressed at low levels in normal adult liver, but is induced during rapid liver growth and dedifferentiation.[1] Like MAT1A, MAT2A expression is regulated both transcriptionally and post-transcriptionally. We identified Sp1, c-MYB, nuclear factor-κB (NF-κB), and AP-1 as participants in *MAT2A* transcriptional up-regulation in HCC.[20–22] Hypoxia-inducible factor factor-1α (HIF-1α), activated in HCC, binds to a consensus binding site in the *MAT2A* promoter and activates transcription in hepatoma cells.[23] Hepatitis B X protein also activates *MAT2A* gene transcription by facilitating NF-κB and CREB binding to the *MAT2A* promoter, contributing to induction of MAT2A in HBV-associated HCC.[24] Promoter methylation and histone acetylation also regulate human *MAT2A* transcription.[25,26]

Post-transcriptionally, *MAT2A* mRNA level is regulated by HuR and methylated-HuR.[15] HuR is a ubiquitously expressed mRNA binding protein known to stabilize its target mRNAs, whereas methylated-HuR exerts the opposite effect.[15] Interestingly, during hepatocyte de-differentiation and in HCC, there is a switch from methylated-HuR to HuR binding of the 3′-UTR of *MAT2A*, resulting in increased *MAT2A* mRNA level.[15] Several miRNAs also regulate MAT2A post-transcriptionally. MiR-21–3p, induced by the anticancer drug berberine, was shown to suppress *MAT2A* mRNA level in HepG2 cells leading to growth arrest and apoptosis.[27] Recently, both miR-34a and miR-34b were shown to directly target the *MAT2A* 3′UTR, downregulating MAT2A expression in colon, prostate, and pancreas cancer cells.[28] Both miR-34a and miR-34b are tumor suppressor miRNAs and the former is downregulated in HCC,[29] which may contribute to MAT2A induction in HCC.

MATα2, the protein encoded by *MAT2A*, is also regulated via multiple post-translational modifications (PTMs). MATα2 is stabilized by sumoylation at several lysine residues, K340, K372, and K394.[30] Sumoylation also enhances MATα2's interaction with an important pro-survival protein, B-cell lymphoma 2 (BCL-2), leading to BCL-2 stabilization.[30] Other HCC-relevant PTMs of MATα2 are acetylation and ubiquitylation. MATα2 can be acetylated at K81 by P300 (E1A binding protein), which promotes subsequent ubiquitin protein ligase E3 component n-recognin 4-mediated ubiquitylation/degradation.[31] Deacetylation by histone deacetylase 3 (HDAC3) stabilizes MATα2. Interestingly, folate deprivation upregulated MATα2 K81 acetylation, destabilized MATα2 and reduced cell growth.[31] In HCC, a decrease in MATα2 K81 acetylation is associated with increased expression of HDAC3 compared to normal tissue, underscoring relevance of this PTM to liver cancer.[31]

MAT2B

Less is known about MAT2B regulation. At the transcriptional level, tumor necrosis factor α (TNF-α) induces V1 or MATβ expression (but not V2) by mechanisms involving AP-1 and NF-κB.[8] In liver cancer cells, leptin increases, while SAMe inhibits *MAT2B* promoter activity and expression via ERK and AKT signaling mechanisms.[32] We also demonstrated that sirtuin 1 (SIRT1) can activate *MAT2B* transcriptionally.[33] Post-transcriptionally, HuR stabilizes *MAT2B* mRNA,[33] whereas miR-21–3p directly targets *MAT2B* 3′UTR to destabilize its mRNA.[27] At the protein level, MATβ stability is affected by several protein interactions. MATβ-MATα2 interaction is suspected to stabilize these two proteins as overexpression of MAT2B raised MATα2 levels.[34] We confirmed this, as knockdown of endogenous MAT2A or MAT2B lowered protein level of the other in multiple human cancer cell lines.[28] This finding helps explain why these two proteins are often simultaneously induced. MATβ also interacts with HuR and SIRT1, and resveratrol enhances these interactions by stabilizing them.[33] Finally, both variants of MAT2B also interact with G-protein-coupled receptor kinase-interacting protein 1 (GIT1) to form a scaffold complex. This interaction stabilizes all participants and activates RAS-RAF-MEK-ERK signaling in liver and colon cancer cells.[35,36] Table 2 summarizes regulation and dysregulation of the three MAT genes.

Consequences of MAT gene dysregulation

MAT1A

MAT1A expression and MATI/III activity falls in chronic liver disease.[1] Epigenetic hypermethylation of promoter and coding regions is attributed to this decline in gene expression and activity. This explains observed impairment of methionine clearance and reduced hepatic GSH level in liver disease patients, since MATI/III are largely responsible for methionine catabolism and SAMe is a key precursor for liver GSH, respectively.[1] In HCC, MAT1A is often silenced and low MAT1A expression correlates with poor prognosis.[10,37] The *Mat1a*-knockout (KO) mouse model (*Mat1a*-KO) has revealed consequences of chronic hepatic SAMe deficiency and provided insight on how loss of MAT1A can lead to HCC development. *Mat1a*-KO mice have reduced hepatic SAMe and GSH levels, consistent with what's known about the kinetic and regulatory differences between the MAT isoenzymes and the importance of

Table 2. Regulatory mechanisms of human MAT genes and proteins.

MAT gene	Expression pattern in cancers	Transcriptional regulation	Post-transcriptional regulation	Post-translational regulation
MAT1A	Downregulated in hepatocellular carcinoma and cholangiocarcinoma	Silenced by promoter and coding region hypermethylation; increased by glucocorticoids and C/EBP; downregulated by c-MYC, MAFG and c-MAF	Inhibited by AUF1, miR-485-3p, miR-495, miR-664 by binding to *MAT1A* 3′-UTR	Inactivated by covalent modification of cysteine 121, which can be reversed by GSH
MAT2A	Upregulated in liver, colon, gastric, breast, pancreas, prostate cancers	Induced by promoter hypomethylation; increased by Sp1, c-MYB, NF-κB, AP-1, HIF-1α, and HBx; suppressed by SAMe	Increased by HuR; inhibited by methylated-HuR, miR-21-3p, miR-34a, miR-34b binding to *MAT2A* 3′-UTR	Stabilized by sumoylation at K340, 373, 394, and interaction with MATβ; destabilized by acetylation at K81
MAT2B	Upregulated in liver, colon, pancreas and prostate cancers	V1 (but not V2) is induced by AP-1, NF-κB, and SIRT1; inhibited by SAMe	Increased by HuR; inhibited by miR-21-3p binding to *MAT2B* 3′-UTR	Stabilized by interaction with MATα2 and GIT1

MAT: methionine adenosyltransferase.

SAMe as a precursor of GSH in the liver.[38] Although KO livers appear normal at three months, liver weights are greater and KO mice are sensitized to develop fatty liver when challenged with a choline-deficient diet for six days.[38] KO mice have increased activity of the cytochrome P450 2E1 (CYP2E1) enzyme, which facilitates release of reactive oxygen species (ROS) during hepatotoxin metabolism.[39] On a normal diet, KO mice spontaneously develop steatohepatitis by 8 months and HCC by 18 months.[38,40] Mitochondrial dysfunction is also evident in *Mat1a*-KO mice; MAT1A deficiency depletes the mitochondrial chaperone, prohibitin 1 (PHB1), leading to increased propensity for injury, mitochondrial damage, and oxidative stress.[41]

While increased ROS sensitizes KO mice to liver injury, multiple dysregulated pathways are implicated in HCC development. These pathways include expansion of progenitor cells, some of which are cancer stem cells in aging KO mice,[42] increased genomic instability due to decreased protein level of apurinic/apyrimidinic endonuclease 1,[43] and uncontrolled extracellular signal-regulated kinases (ERK) activation due to decreased stability of dual-specificity mitogen-activated protein kinases (MAPK) phosphatase.[44] Other pathways include: abnormal LKB1/AMPK signaling, (which behaves as an oncogenic pathway in liver),[45,46] reduced protein level of PHB1 (a mitochondrial chaperone protein that may also function as a tumor suppressor in hepatocytes and bile duct epithelial cells),[41,47] and increased expression of ubiquitin-conjugating enzyme 9 (UBC9) (the sole E2 enzyme in protein sumoylation that is often induced in cancer).[48] Among these dysregulated pathways, restoration of hepatic SAMe level normalized ERK activity and reduced UBC9 expression.[44,48]

Though SAMe deficiency may contribute to dysregulation of many signaling pathways, we recently reported that MATα1 can act as a transcription co-factor that interacts with other E-box binding regulatory proteins, such as c-MYC.[2,47] We identified c-MYC, MAFG, c-MAF, MAX network transcriptional repressor (MNT), PHB1, and MYC associated factor X (MAX) as MATα1 interacting proteins that differentially regulate gene expression at the E-box (5′-CACGTG-3′), with c-MYC/MAFG/c-MAF serving as E-box activators, and PHB1 and MATα1 as repressors.[2,47] It is well known that c-MYC and MNT compete for heterodimerization with MAX for E-box binding in opposite ways.[49] Our recent studies demonstrated that MATα1 can also heterodimerize with MAX, and bind to the E-box to repress E-box-driven promoter activity.[47] Interestingly, while *c-MYC* possesses an E-box that acts as an enhancer element, *MAT1A* has a repressor E-box. Thus, MAT1A and c-MYC exert reciprocal regulation against each other in part through this mechanism.[2] MAT1A can regulate gene expression through epigenetics (i.e. *LIN28B* promoter methylation mentioned above), but its repressive effect on c-MYC expression occurs despite inhibition of DNA methylation or histone 3 lysine 27 trimethylation (H3K27me3, a transcriptional repressor).[2] This supports the notion that MATα1 can also regulate gene expression as a transcription co-factor (it is unable to bind to the E-box by itself), independent of epigenetics. MAT1A is expressed at high levels in normal hepatocytes and bile duct epithelial cells and is repressed in CCA, where c-MYC, MAFG, and c-MAF are induced. These changes favor c-MYC induction as well as CCA proliferation because both effects are suppressed by MAT1A overexpression.[2] Together, loss of MAT1A can result in altered liver signaling pathways due to chronically low SAMe levels and/or loss of MATα1-regulated gene expression, leading to development of liver pathology including HCC and CCA.

MAT2A

In HCC, the fall in MAT1A expression is often accompanied by MAT2A induction.[10,50] Increased MAT2A expression provides cancer cells a dual growth/survival benefit. Due to kinetic differences and regulatory properties discussed above, a switch from MAT1A to MAT2A expression results in lower steady state SAMe level and favors proliferative signaling in the liver.[50,51] Indeed, quiescent

hepatocytes have high SAMe levels and a fall in SAMe level is needed for hepatocyte proliferation in conditions such as post-partial hepatectomy.[1] SAMe's inhibitory effect on hepatocyte growth factor (HGF),[52] required for liver regeneration, is one proposed mechanism that explains this observation. However, in cancer cells where MAT1A is not expressed, an increase in MAT2A crucially supplies SAMe for polyamine biosynthesis.[22] Similarly, silencing *MAT2A* prevents leptin's pro-survival signaling by decreasing intracellular SAMe and limiting polyamine biosynthesis.[32] Cross-talk between MAT2A and polyamine biosynthesis also occurs in both liver and colon cancer cells.[22] Induction of MAT2A enhances polyamine biosynthesis and growth, while increase in polyamines promotes *MAT2A* transcription by a feed-forward mechanism involving AP-1.[22]

Increased MAT2A expression can bestow a survival benefit by enhancing BCL-2 expression.[30] At least two mechanisms are involved. First, MATα2 can act as a transcription factor that directly binds to the *BCL-2* promoter and activates promoter activity.[30] Second, MATα2 can physically interact with BCL-2 protein to enhance BCL-2 stability.[30] These MATα2 mechanisms require sumoylation at critical lysine residues, which stabilize MATα2[30] and may enhance nuclear targeting and DNA *trans*-activating activity (topics for future investigation). SUMO-stabilized MATα2 confers chemo-resistance to liver and colon cancer cells by preventing apoptosis mediated by 5-fluorouracil (5-FU).[30]

Recently, we reported that increased MAT2A expression in cancer cells enhances tumor migration and invasion.[28] This is largely because increased MATα2 stabilizes MATβ, which we believe is the critical mediator in these processes.

MAT2B

As mentioned above, MAT2B and MAT2A expression often simultaneously increase, which is unsurprising given their frequent interaction and co-stabilizing effect.[28] However, other mechanisms may also be involved as their mRNA levels are often altered in parallel (such as in HCC).[8] Some of these shared mechanisms include AP-1 and NF-κB, both transcription activators, and HuR, which stabilizes both mRNAs.

Similar to MAT2A, increased MAT2B confers a growth advantage; silencing either gene can lead to cancer cell apoptosis.[8,24] One of MAT2B's effects on growth is attributed to its ability to lower SAMe level to steady-state concentration when overexpressed.[53] More recent studies found MAT2B regulates much more than MATII enzyme activity. To better understand the functions of MAT2B variants, we identified their interactomes[54] and functionally verified the importance of two of them in growth. First is the interaction between MAT2B variants and HuR.[54] We found that when either of the MAT2B variants is overexpressed, cytosolic HuR content increased, leading to increased mRNA levels of cyclin D1 and cyclin A (known targets of HuR) and increased growth.[54] The opposite occurred when MAT2B variants were silenced.[54] Second, the interaction between MAT2B variants and GIT1 form a scaffold complex that efficiently binds and activates MAPK components, mitogen-activated protein kinase (MEK), and ERK.[35,36] Silencing either MAT2BV1, V2, or GIT1 blocks ERK activation.[35] In the classical MAPK activation pathway, signal transduction from RAF kinases to MEK causes phosphorylation and activation.[55] MAT2B variants-GIT1 complex activates and recruits C-RAF and B-RAF to MEK in liver cancer cell lines, promoting MEK activation.[36] B-RAF and C-RAF also form homodimers and heterodimers that enhance activity compared to monomeric states.[56] The MAT2B variants-GIT1 complex also induces heterodimerization between B-RAF and C-RAF,[36] thereby amplifying the MEK signal. Finally, MAT2B variants interact, stabilize, and activate RAS, located upstream of RAF kinases.[36] Thus, the MAT2B variants-GIT1 complex interacts and activates all components of RAS-RAF-MEK-ERK signaling – a pathway often dysregulated in cancers. Of note, the effects of MAT2B variants on this signaling pathway occurred independent of MATα2 in these studies, as the overexpression vectors used included a tag that prevents MATα2 interaction. While MAT2B variants can exert pro-tumorigenic effect without MATα2, we suspect MATα2 requires MATβ to induce ERK (this warrants further investigation).

MAT genes in other cancers

Although most studies reporting MAT gene involvement in cancer focus on liver, there are increasing reports that MAT2A and MAT2B are also dysregulated in other cancers, namely: colon, gastric, breast, pancreatic, and prostate cancer.

In colon cancer, *MAT2A* mRNA levels and MATα2 protein levels are elevated, mirroring expression in liver cancer.[57] In contrast to liver cancer, however, *MAT2B* mRNA levels remain unchanged but protein levels are increased.[35,57] Four signaling pathways are recognized in colorectal carcinogenesis: Wnt, K-RAS, transforming growth factor β, and p53,[58] with mutations in these pathways leading to inactivation of tumor suppressor function or activation of proto-oncogenes. Additionally, epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and leptin are three well-known growth factors implicated in colon cancer growth and invasion.[59,60] All three growth factors induce MAT2A gene expression through transcriptional up-regulation, and promote growth by via endogenous production of SAMe and polyamines; exogenous SAMe and its metabolite methylthioadenosine (MTA) prevent MAT2A induction by these growth factors.[57] Mitogens such as IGF-1 are thought to up-regulate MAT2A expression at the transcriptional level, through increased binding of transcription factors to enhancer elements including AP-1 and NF-κB.[61]

There are shared pathways in which MAT2A is implicated in both colon and liver carcinogenesis. Crosstalk between MAT2A and polyamines, as well as BCL-2 regulation occurs in both colon and liver cancer cells. Similarly, there are significant parallels between MAT2B's role in liver and colon cancer. MAT2B and GIT1 are over-expressed in both cancers, and regulate RAS-RAF-MEK-ERK signaling similarly in both cell types.[35,36] Mutations in this signaling pathway are well known in many cancers, and targeting

RAS-RAF-MEK-ERK has been the subject of intense research.[62]

MAT2A upregulation also occurs in gastric cancer. *MAT2A* mRNA levels are significantly higher in gastric cancer patients than in corresponding non-tumor tissues and are correlated with high tumor classification indicating lymph node metastasis and poor tumor differentiation.[63] Studies also describe the effect of SAMe on c-MYC, H-RAS and tumor-suppressor gene p16 (INK4a) promoter methylation, as well as gastric cancer cell inhibition. SAMe treatment led to heavy promoter methylation, which consequently downregulated mRNA and protein levels, and prevented gastric cancer growth[63] pointing to SAMe supplementation as an attractive therapeutic approach.

Like gastric cancer, tamoxifen-resistant breast cancer tissues (TAMR-MCF-7 cells) exhibited increased basal expression of MAT2A compared to MCF-7 control cells. This was also observed in tamoxifen-resistant human breast cancer tissues compared to tamoxifen-responsive cells. Increased transcription factors NF-κB, AP-1, and NRF2 in cell lines may be attributed to these observations.[64] Furthermore, the effect of SAMe appears to depend on whether it is increased endogenously (i.e. via increased MAT2A expression) or given in pharmacologic amounts. One study showed that a 30% increase in endogenous SAMe level found in TAMR-MCF-7 cells correlated with hypermethylation of the tumor suppressor phosphatase and tensin homolog (PTEN) and reduced PTEN expression that facilitated growth[65]; however, exogenous SAMe (200–500μM, more than 10 times physiological level) induces autophagy and apoptosis in MCF-7 cell lines, respectively.[66] This seemingly contradictory finding can be explained by whether SAMe is varied physiologically or pharmacologically (see below).

The role of MAT genes and SAMe in pancreatic cancer is not well-studied. Interestingly, outside the liver, MAT1A is highly expressed in pancreatic acinar cells[67]; however, *Mat1a* KO mice do not develop pancreatic cancer (Lu SC, unpublished observation). In severe necrotizing pancreatitis models, SAMe levels are 50% decreased and MAT1A protein levels are post-translationally diminished, while MAT2A expression is induced.[67] Recently, we found MAT2A and MAT2B proteins are overexpressed in pancreatic cancer and overexpressing MAT2A induced migration of pancreatic cancer cells, likely due to enhancing MAT2B protein stability and activating ERK.[28] Like colon cancer, miR-34a expression is often downregulated in pancreatic cancer.[68] MAT2A is directly targeted by miR-34a (and miR-34b) to suppress expression and indirectly lower MAT2B protein level by stability reduction.[28]

In prostate cancer, miR-34a expression is also downregulated, facilitating cancer metastasis via miR-34a suppression of cancer stemness.[69] We confirmed that miR-34a and miR-34b also negatively regulate MAT2A and MAT2B at the protein level in prostate cancer cells and overexpressing MAT2A enhanced cancer migration.[28] Consistently, both MAT2A and MAT2B are induced in prostate cancer specimens.[28]

MAT2A and MAT2B are key targets of miR-34a and miR-34b. This is supported by evidence that miR-34a and miR-34b overexpression has minimal to no effect on cancer cell growth, migration, or invasion when either MAT2A or MAT2B is overexpressed.[28] This strengthens support for targeting MAT proteins in cancer treatment development. Table 3 summarizes consequences of MAT genes dysregulation.

Table 3. Consequences of MAT genes dysregulation.

MAT genes	Consequences of dysregulation
MAT1A	• *Mat1a* KO mice have hepatic SAMe and GSH deficiency, increased oxidative stress, and spontaneous development of NASH and HCC • Dysregulated pathways include cancer stem cells, genomic instability, sustained ERK activation, increased LKB1/AMPK, and sumoylation • Effects on gene expression: ○ MAT1A can regulate gene expression via epigenetics (i.e. promoter methylation) and as a transcription co-factor (i.e. heterodimerize with MAX, binds to the E-box and represses E-box dependent genes, such as *c-MYC*)
MAT2A	• Increased MAT2A expression confers cancer cell growth advantage partly by enhancing polyamine biosynthesis, which further induces MAT2A expression • MAT2A overexpression confers cancer cell survival advantage due to MATα2's role as a transcription factor that binds and activates the *BCL-2* promoter • MATα2 physically stabilizes BCL-2 which increases chemoresistance to 5-FU in both liver and colon cancer cell • MAT2A enhances cancer cell migration through stabilizing MAT2B protein
MAT2B	• Increased MAT2B expression: ○ Promotes tumor growth and metastasis ○ Promotes HuR cytosolic content, stabilizing many of its targets that include cyclins to promote growth ○ MAT2B variants and GIT1 form a scaffold complex that interacts and activates RAS-RAF-MEK-ERK signaling in liver and colon cancer cells, promoting growth and metastasis

MAT: methionine adenosyltransferase.

Targeting MAT genes in cancer therapeutics

As our understanding of MAT genes and SAMe's role in cancer pathogenesis evolves, increasing ideas for potential therapeutic interventions surface. Over two decades of research supports SAMe's role as a potential chemopreventive agent for HCC, and emerging evidence now supports its role in treatment of extra-hepatic malignancies. Additionally, work over the last few years has elucidated the role of MAT1A, MAT2A and MAT2B in tumorigenesis and identifying these genes as valuable targets for cancer therapeutic research and development. This section summarizes the role of SAMe in this context and emerging approaches that target MAT genes in cancer therapeutics.

SAMe as a chemopreventive and/or therapeutic agent

As previously mentioned, in human HCC, the MAT1A to MAT2A switch is accompanied by increased MAT2B expression, associated with lower SAMe levels and faster growth.[1] Interestingly, SAMe maintains MAT1A expression and suppresses MAT2A and MAT2B expression.[32,70]

The *Mat1a-KO* mouse model has clearly illustrated the multitude of dysregulated pathways that favor HCC development in the setting of chronic hepatic SAMe depletion. Indeed, SAMe has demonstrated efficacy as a chemopreventive agent against HCC rodent models in multiple studies.[71–73] SAMe's chemopreventative ability is attributed to preventing proto-oncogene promoter hypomethylation in these models, which results in gene induction. Using an orthotopic HCC model where hepatic SAMe level is not altered, however, SAMe dramatically inhibited tumor establishment and growth suggesting other mechanisms also participate, such as inhibiting angiogenesis and inducing apoptosis of liver cancer cells.[74] Interestingly, SAMe treatment post-HCC development was ineffective in tumor growth reduction.[74] This phenomenon was attributed to compensatory induction of glycine N-methyltransferase (GNMT), the liver's dominant methyltransferase, from chronic SAMe administration, preventing hepatic SAMe accumulation.[74] Although hepatic SAMe level consistently increased 10-fold following 24 h of intravenous administration, it was only 30% higher after 24 days of SAMe administration, which is not high enough to induce apoptosis (see below).[74] However, GNMT expression is often downregulated in human HCC,[75] so the verdict is still out whether SAMe should be used therapeutically. One attractive feature of SAMe is that while it is pro-apoptotic in liver cancer cells, it is anti-apoptotic in normal hepatocytes.[76,77] The minimum concentration required for SAMe to induce apoptosis in liver cancer cells after 18 h is 0.5 mM (nearly 10-fold higher than normal hepatic SAMe concentration),[77,78] which explains why a 30% increase in hepatic SAMe level did not result in tumor shrinkage.

SAMe versus MTA

Physiological variation and implications for pharmacologic treatment are two important contexts in which we can better understand differences between SAMe and MTA. SAMe is unstable and spontaneously converts to MTA (1.3% per hour at 37°C, pH 7.0); thus, MTA may mediate SAMe's pharmacologic effect.[57,79] Unlike SAMe, MTA is highly stable, readily traverses the plasma membrane, and inhibits both methylation and polyamine biosynthesis.[1] However, MTA can regenerate SAMe via the methionine salvage pathway, which can be blocked with a MAT inhibitor like cycloleucine.[1] Thus, if the biological effect is very rapid (so conversion to MTA is minimal), or associated with an increase in methylation, and/or MTA's effect is blocked by cycloleucine, then SAMe is the active molecule. On the other hand, if the outcome is not blocked by cycloleucine or is associated with a fall in methylation, then MTA is the active molecule. This is illustrated by SAMe and MTA's inhibitory effect on lipopolysaccharide (LPS)-induced expression of proinflammatory cytokines where MTA was the active compound that blocked the LPS-mediated increase in methylated histone 3 lysine 4 (H3K4) associated with these promoters.[79] In contrast, MTA's ability to prevent the fall in Apurinic/Apyrimidinic Endonuclease 1 (a critical protein involved in DNA repair) protein level during culture of hepatocytes was blocked by cycloleucine, which means MTA's effect was mediated by SAMe.[43] In *Mat1a-KO* mice, abnormalities corrected after normalization of hepatic SAMe level with supplemental administration suggest these are due to SAMe deficiency as MTA levels are not affected in the KO mice.[38]

Similar to SAMe, MTA also inhibits HCC cell growth and prevents development of HCC in rats.[71–73] In hepatoma cells, SAMe and MTA prevented leptin's ability to induce MAT2A and MAT2B, which are required for growth.[32]

Both SAMe and MTA affect apoptosis. Interestingly, both are anti-apoptotic in normal hepatocytes but pro-apoptotic in liver cancer cells.[76] They selectively induce Bcl-x_s, a pro-apoptotic protein, in liver cancer cells by increasing alternative splicing of Bcl-x.[77] SAMe and MTA also induce apoptosis in colon cancer cells, but not in NCM460 cell line – an immortalized cell line derived from normal human colonocytes.[80] The mechanism of apoptosis is completely different from liver cancer cells.[81] SAMe and MTA lowered expression of cellular FLICE inhibitory protein, an anti-apoptotic protein that targets procaspase 8, and had no influence on Bcl-x_S expression.[81]

There is also substantial evidence to support SAMe's role as a potential chemopreventive agent in colon cancer. Chronic inflammation is an underlying risk factor for colon cancer, and mouse models demonstrate that TNF-α plays a critical role in development of inflammation-induced colon cancer.[82,83] Like others, we have shown that SAMe and MTA can reduce the LPS-mediated increase in TNF-α and iNOS expression in mouse macrophages *in vitro* and mouse livers *in vivo*.[79,84] In addition to altering inflammation, both SAMe and MTA reduce the mitogenic effects of IGF-1, EGF, and leptin in colon cancer cells, as previously mentioned.[57] In the colitis-associated colon cancer models, treatment with SAMe and MTA lowered total tumor load by suppressing multiple oncogenic pathways that include NF-κB activation, IL-6 signaling, and β-catenin.[85] The mechanism for lowering β-catenin was further examined in both colon and liver cancer cells. SAMe and MTA excluded β-catenin from the nuclear compartment in cells with constitutively active β-catenin, but accelerated β-catenin degradation by a glycogen synthase kinase 3-β-dependent mechanism in cells with wild type β-catenin.[86] This ability to suppress β-catenin regardless of whether this pathway is aberrantly induced further supports their use as chemopreventive agents in cancers where aberrant β-catenin pathway is a prominent feature. Recently, we showed that SAMe and MTA can raise the expression of miR-34a and miR-34b and prevent colon cancer liver metastasis in a mouse model of metastatic colon cancer.[28]

SAMe in other cancers

In gastric cancer, SAMe treatment inhibits the growth of gastric cancer cell lines, SGC-7901 and MKN-45, and significantly decreased mRNA expression of c-MYC and urokinase type plasminogen activator (uPA).[63] These studies

were confirmed using *in vivo* xenograft experiments.[63] In breast cancer cell lines, the role of SAMe as an adjunct to existing chemotherapeutic therapies is currently being explored. When SAMe is combined with Doxorubicin, the anti-proliferative effect is strongly synergistic in the hormone-dependent CG5 and MCF-7 human breast cancer cell lines. This synergy is attributed to apoptosis induction with observed activation of caspase 3 and 8; this effect was also observed in hormone-independent MDA-MB 231 cells, but less so, suggesting an additive rather than synergistic effect.[87] Additionally, SAMe may play a role in cancer epigenetic therapy. 5-Azacytidine, a chemotherapeutic agent used in myelodysplastic disorders, and its deoxy-analog, 5-aza-2′-deoxycytidine are known to induce cancer cell invasiveness and methylated prometastatic genes by DNA demethylation.[88] As a methyl donor, SAMe may block the adverse demethylating activity of 5-aza-2′-deoxycytidine while maintaining growth suppression effects. Additionally, SAMe demonstrated reversal of global- and gene-specific demethylation induced by 5-aza-2′-deoxycytidine preventing cell invasiveness in breast cancer cell lines, while still augmenting growth inhibitory effects of 5-aza-2′-deoxycytidine.[88] Thus, SAMe is a potential candidate for use in combination therapy aimed at targeting DNA methylation machinery as a new strategy for epigenetic cancer therapy.

Targeting miRNAs that regulate MATs

Several miRNAs regulate MAT1A, MAT2A, and MAT2B. We have demonstrated that targeting the three miRNAs induced in HCC, miR-485–3p, miR-495, and miR-664, is a viable strategy for HCC treatment using the orthotopic HCC model.[17] Indeed, when these miRNAs are knocked down, we observe a recovery in *MAT1A* expression, growth inhibition, and increased apoptosis in HCC *in vivo*.[17] MiR-34a and miR-34b, however, negatively regulate MAT2A/MAT2B expression; thus, one may strategically increase expression of these miRNAs to achieve gene suppression.[28] Similarly, berberine was shown to increase the expression of miR-21–3p, which target MAT2A and MAT2B in a human hepatoma cell line.[27] As technology evolves, targeting miRNAs that control gene expression will become one of the best approaches to cancer treatment.[89]

Other approaches that target MATs

Recently, a novel allosteric inhibitor of MAT2A protein that overlaps with the MAT2B binding site demonstrated promise in cancer treatment.[90] Interrupting interaction between MAT2B variants and GIT1 is likely to inhibit RAS-RAF-MEK-ERK signaling, and thus may be another promising therapeutic approach.

Conclusions and future directions

In conclusion, increasing evidence suggests that MAT genes are dysregulated in many cancers and these pathways play critical roles in tumorigenesis. Targeting MAT gene dysregulation with chemicals, small molecules that block interactions or miRNAs that control expression may prove effective strategies in future cancer therapy research and development. While much advance has been made on understanding the role of MAT genes in various cancers, many questions remain for future investigation. For instance, MATα1 and MATα2 share high homology yet one is a tumor suppressor and the other an oncoprotein in the liver. The underlying molecular mechanisms for this distinct behavior are unclear. In addition, we have only begun to unravel how these MAT proteins regulate gene expression transcriptionally and proteins functionally. Future investigations should include identifying genes that are regulated by these MAT proteins, either epigenetically or as transcription factors or co-factors. Finally, a better characterization of the MAT proteins interactomes is likely to reveal unknown functions of these proteins that may provide more targets for designing therapy.

Authors' contributions: LM, DA and SL wrote the paper, JM provided intellectual input.

DECLARATION OF CONFLICTING INTERESTS

JMM is a consultant for Abbott, Galmed and OWL; the other authors have nothing to declare

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