Molecular Genetics and Metabolism Reports 15 (2018) 15–21

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

S-adenosylhomocysteine hydrolase over-expression does not alter S-adenosylmethionine or S-adenosylhomocysteine levels in CBS deficient mice

Hyung-Ok Lee[a], Liqun Wang[a], Yin-Ming Kuo[b], Andrew J. Andrews[b], Sapna Gupta[a], Warren D. Kruger[a,*]

[a] Cancer Biology Program, Fox Chase Cancer Center, Philadelphia, PA, USA
[b] Cancer Epigenetics Program, Fox Chase Cancer Center, Philadelphia, PA, USA

ARTICLE INFO

Keywords:
Metabolism
Methionine
Transgenic

ABSTRACT

Elevated plasma total homocysteine (tHcy) is associated with a number of human diseases including coronary artery disease, stroke, osteoporosis and dementia. It is highly correlated with intracellular S-adenosylhomocysteine (SAH). Since SAH is a strong inhibitor of methyl-transfer reactions involving the methyl-donor S-adenosylmethionine (SAM), elevation in SAH could be an explanation for the wide association of tHcy and human disease. Here, we have created a transgenic mouse (*Tg-hAHCY*) that expresses human S-adenosylhomocysteine hydrolase (*AHCY*) from a zinc-inducible promoter in the liver and kidney. Protein analysis shows that human AHCY is expressed well in both liver and kidney, but elevated AHCY enzyme activity (131% increase) is only detected in the kidney due to the high levels of endogenous mouse AHCY expression in liver. *Tg-hAHCY* mice were crossed with mice lacking cystathionine β-synthase activity (*Tg-I278T Cbs*$^{-/-}$) to explore the effect to AHCY overexpression in the context of elevated serum tHcy and elevated tissue SAM and SAH. Overexpression of AHCY had no significant effect on the phenotypes of *Tg-I278T Cbs*$^{-/-}$ mice or any effect on the steady state concentrations of methionine, total homocysteine, SAM, SAH, and SAM/SAH ratio in the liver and kidney. Furthermore, enhanced AHCY activity did not lower serum and tissue tHcy or methionine levels. Our data suggests that enhancing AHCY activity does not alter the distribution of methionine recycling metabolites, even when they are greatly elevated by *Cbs* mutations.

1. Introduction

Elevated plasma total homocysteine (tHcy), termed hyperhomocysteinemia (HHcy), is associated prospectively with increased incidence of mortality for many human diseases including coronary heart disease, stroke, dementia, Alzheimer's disease, diabetic retinopathy, osteoporosis, cancer, congenital birth defects and steatosis of the liver [1–9]. The number and variety of diseases associated with elevated tHcy suggests that it may affect very basic cellular functions. Homocysteine (Hcy) is a non-protein incorporated amino acid that is part of the methionine-recycling pathway (Fig. 1). It is catabolized by the action of cystathionine β-synthase (CBS), which initiates the conversion of Hcy to cysteine via the transsulfuration pathway. Hcy is formed via the hydrolysis of S-adenosylhomocysteine (SAH), which is a product of methyl-transfer reactions involving the methyl-donor S-adenosylmethionine (SAM). SAM is the key one-carbon donor in almost all biological methylation reactions. In the human genome, there are at least 53 known or suspected SAM-dependent protein methyltransferases, 13 SAM-dependent RNA methyltransferases, and 5 SAM-dependent DNA methyltransferases (http://www.hprd.org/). Because the chemical structure of SAM and SAH are nearly identical, SAH can bind to the active site of methyltransferase enzymes and act as a potent competitive inhibitor of those enzymes. Kinetic studies have shown that many DNA and protein methyltransferases bind SAH as well, or even slightly tighter than SAM [10–14]. Thus, the ratio of SAM to SAH inside the cell is thought to be critical for proper regulation of methyl transfer reactions.

Elevated tHcy in serum or plasma is strongly correlated with increased tissue SAH and a reduction in tissue SAM/SAH ratio. In plasma, SAH and SAM are present at approximately 1/500th the concentration

Abbreviations: SAH, S-adenosyl homocysteine; SAM, S-adenosyl methionine; CBS, cystathionine beta synthase; HHcy, hyperhomocysteinemia; Hcy, homocysteine; tHcy, total homocysteine; *Cbs*$^{-}$, CBS knockout allele; *AHCY*, S-adenosylhomocysteine hydrolase; *Tg-I278T*, transgene human CBS containing the I278T mutation; Zn, zinc water; HA, hemagglutinin; CMC, carboxymethylcellulose; Met, methionine

* Corresponding author at: Cancer Biology Program, Fox Chase Cancer Program, 333 Cottman Avenue, Philadelphia, PA 19111, USA.
E-mail addresses: Hyung-Ok.Lee@fccc.edu (H.-O. Lee), Liqun.Wang@fccc.edu (L. Wang), Yin-Ming.Kuo@fccc.edu (Y.-M. Kuo), Andrew.Andrews@fccc.edu (A.J. Andrews), Sapna.Gupta@fccc.edu (S. Gupta), Warren.Kruger@fccc.edu (W.D. Kruger).

https://doi.org/10.1016/j.ymgmr.2018.01.002
Received 21 December 2017; Received in revised form 8 January 2018; Accepted 8 January 2018
Available online 12 January 2018

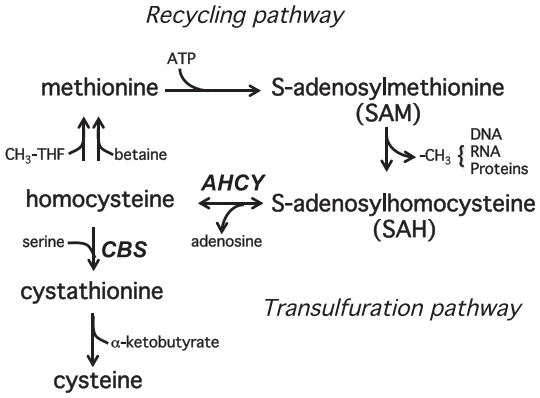

Fig. 1. Methionine metabolism. Figure shows both the methionine recycling pathway and the transsulfuration pathway. The key enzymes for this manuscript, CBS and AHCY are shown in bold by their respective reactions.

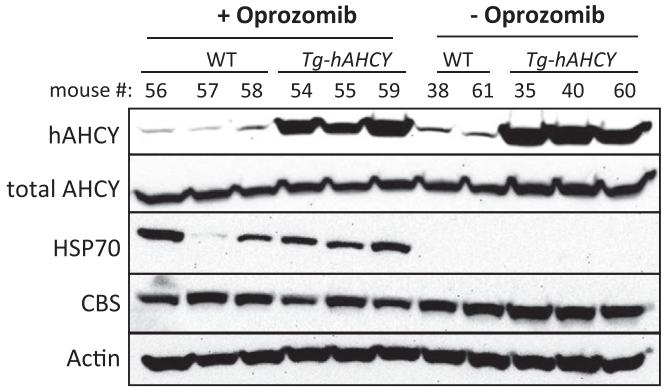

Fig. 3. Effect of proteasome inhibitor on AHCY protein levels. Western blot analysis of transgene negative and *Tg-hAHCY* liver lysates of oprozomib treated mice. Blots were probed with the indicated antibodies.

of tHcy, about 20–80 nM versus 5–15 μM [15]. However, in tissue, such as liver or kidney, the concentration of all four methionine recycling metabolites (methionine, SAM, SAH and homocysteine) are within the same order of magnitude [16–19]. The reason for this is that homocysteine and methionine are membrane permeable, but SAM and SAH are not [20]. Therefore, for a cell to get rid of excess intracellular SAH, it must first convert it to Hcy and only then can it be secreted into the blood. A variety of cellular, animal, and human data supports the idea that plasma tHcy and intracellular SAH levels are strongly correlated [19,21–24].

SAH is converted to Hcy by the enzyme S-adenosylhomocysteine hydrolase, which is encoded by the *AHCY* gene. This is a highly evolutionarily conserved enzyme with 71% amino acid identity between the *H. sapiens* and *S. cerevisiae* protein sequences. AHCY catalyzes the hydrolysis of SAH to homocysteine and adenosine. In vitro this reaction is entirely reversible, and the chemical equilibrium constant actually favors the reverse reation, i.e., the formation of SAH from homocysteine and adenosine. However, in vivo the reaction is driven in the forward direction due to the action of adenosine kinase, which keeps the intracellular concentration of adenosine quite low [20,25]. Loss of SAH hydrolase activity leads to an accumulation of SAH and causes developmental and aging phenotypes in diverse organisms including *D. melanogaster*, *A. thaliana*, *M. musculus*, and *H. sapiens* [26–29].

In this study, we have engineered a mouse that ectopically expresses human *AHCY* from a zinc-inducible transgene to see if increasing AHCY enzyme level alters SAM/SAH homeostasis in mice that have greatly elevated serum tHcy due to a mutation in the *Cbs* gene. Our results suggest that increasing AHCY activity does not alter SAM/SAH homoestasis.

2. Methods

2.1. Plasmid construction

In this study, we generated two recombinant plasmids for injection, one containing the mouse *Ahcy* gene (*pTg-mAhcy*) and one the human *AHCY* gene (*pTg-hAHCY*). For the p*Tg-mAhcy* construction, we first performed PCR amplification of the mouse *Ahcy* ORF (Dharmacon cloneID: 424197) using a 5′ primer containing a hemagglutinin (HA) epitope tag (HA-mAhcy-F) and a 3′ primer containing the stop codon (HA-mAHCY-R). The PCR product was cloned into *PCR2.1* and the lack of mutation was confirmed by sequencing. *PCR2.1:mAHCY* was then digested with *EcoR1*, and the insert was then cloned into the *MfeI* site of plasmid *pUC:MT-I:MfeI* [30]. The resultant plasmid, *PUC:MT-I:mAhcy*,

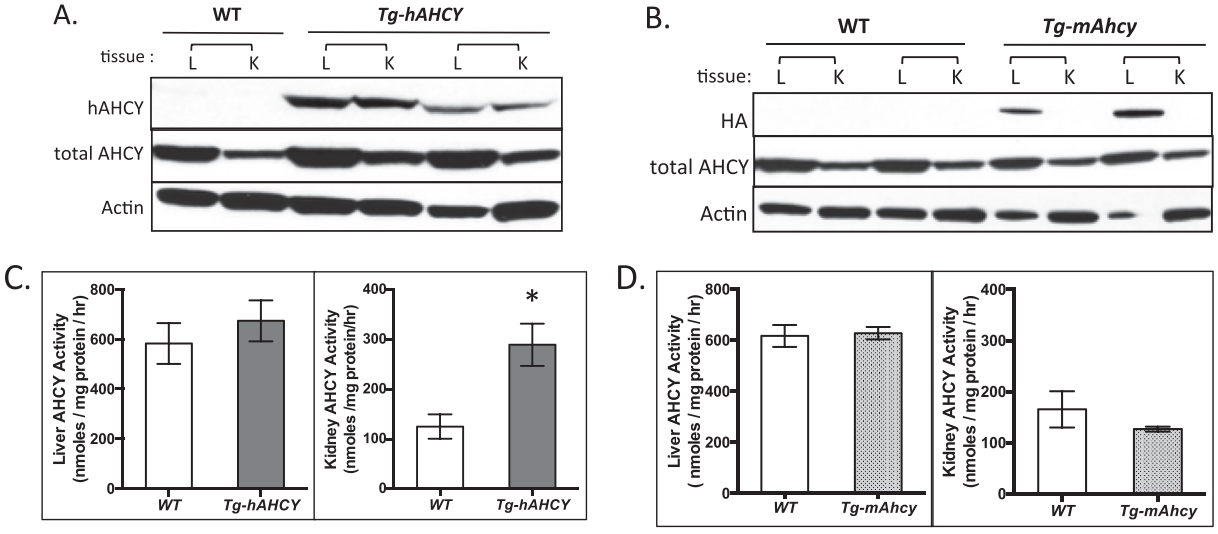

Fig. 2. AHCY expression and activity. (A) Western blot analysis of *Tg-hAHCY* liver and kidney lysates from zinc-induced animals. Lanes labeled WT are from non-transgenic siblings. Top row shows level of human AHCY as determined by human specific antibody. Middle row shows total AHCY as determined by anti-body that recognizes both species. (B) Western analysis of *Tg-mAhcy* liver and kidney. Top row is probed with anti-HA antibody that only recognizes mAHCY expressed from the transgene, middle row shows total AHCY. (C) AHCY enzyme activity in liver (n = 7) and kidney (n = 4) from *Tg-hAHCY* mice and transgene negative controls. (D) AHCY enzyme activity in liver (n = 3) and kidney (n = 4) from *Tg-mAhcy* mice and transgene negative controls. Error bars show SEM. Asterisk indicates $P < 0.05$.