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COMMENTARY

tRNA synthase suppression activates *de novo* cysteine synthesis to compensate for cystine and glutathione deprivation during ferroptosis

Kenichi Shimada[a] and Brent R. Stockwell[a,b]

[a]Department of Biological Sciences, 550 West 120th Street, Columbia University, New York, NY, USA; [b]Department of Chemistry, Howard Hughes Medical Institute, 1208 Northwest Corner Building, MC4846, 550 West 120th Street, Columbia University, New York, NY, USA

ABSTRACT

Glutathione is a major endogenous reducing agent in cells, and cysteine is a limiting factor in glutathione synthesis. Cysteine is obtained by uptake or biosynthesis, and mammalian cells often rely on either one or the other pathway. Because of the scarcity of glutathione, blockade of cysteine uptake causes oxidative cell death known as ferroptosis. A new study suggests that tRNA synthetase suppression activates the endogenous biosynthesis of cysteine, compensates such cysteine loss, and thus makes cells resistant to ferroptosis.

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Defining the connections between metabolic pathways may illuminate therapeutic strategies for treating dysregulated metabolism, tumors, and degenerative disease. Glutathione is a major endogenous reducing agent that protects cells from oxidative stress. Inhibition of glutathione synthesis depletes the glutathione pool in cells; for example, buthionine sulphoximine (BSO) inhibits glutamate-cysteine ligase, the first step in glutathione synthesis, and induces oxidative stress.[1] Glutathione is a linear tripeptide, consisting of glutamate, glycine, and cysteine. Of the 3 precursor amino acids, cysteine contributes primarily to the cofactor's reduction potential—its thiol group acts as an electron donor. Glutathione-dependent oxidoreductases, such as glutathione peroxidases (GPXs), transfer 2 electrons from glutathione to effect substrate reduction. Moreover, cysteine is a limiting factor for glutathione biosynthesis and a decrease in cysteine abundance leads to glutathione depletion.

Cysteine can be obtained by mammalian cells in 2 ways. First, the cells can obtain cysteine by importing cystine, the oxidized disulfide of cysteine, via the cystine-glutamate antiporter system x_c^-.[2] Mammalian cells can also synthesize cysteine *de novo* utilizing 2 amino acids, methionine and serine in a process known as the transsulfuration pathway.[3] When cells rely on cystine uptake via system x_c^- as the primary source of cysteine, inhibition of system x_c^- causes depletion of cysteine, which subsequently depletes glutathione and can induce oxidative stress and subsequent cell death through a regulated, non-apoptotic form of cell death termed ferroptosis.[4,5] This is the lethal mechanism that ensues after pharmacologic inhibition of system x_c^- by the neurotransmitter glutamate or the small molecule erastin. This process is also relevant to some brain and kidney pathologies.[4,5] It has been found that glutathione depletion inactivates glutathione peroxidase 4 (GPX4), a critical cellular antioxidant enzyme that detoxifies lipid hydroperoxides.[6] Inhibition of GPX4 enzymatic activity allows accumulation of overwhelming amounts of lipid peroxides, leading to ferroptosis.

Recently, genome-wide siRNA screening for suppressors of ferroptosis revealed that knockdown of cysteinyl-tRNA synthetase, encoded by the *CARS* gene, inhibits erastin-induced ferroptosis in multiple human and rat cell lines.[7] Although erastin- or glutamate-induced lethality was suppressed by *CARS* knockdown, other classes of ferroptosis-inducing agents, such as inhibitors of glutathione synthesis (*e.g.*, BSO) or GPX4 enzymatic activity, were not suppressed by *CARS* knockdown (Fig. 1). This indicates that *CARS* knockdown specifically interferes with ferroptosis induced by cysteine deprivation caused by erastin and glutamate. In fact, metabolomic profiling revealed that *CARS* knockdown increases cysteine levels in cells. Although the abundance of glutathione itself does not change upon *CARS* knockdown, the level of cysteinyl glutathione disulfide (CSSG) increases; CSSG may then be reduced to form cysteine and glutathione. Thus, an intriguing hypothesis is that *CARS* knockdown increases cysteine abundance and thus glutathione synthesis; in addition, excess glutathione may be stored as CSSG, which is reduced to recover glutathione as needed. This view that glutathione abundance is increased by *CARS* knockdown is supported by the fact that *CARS* knockdown partially suppressed glutathione depletion upon erastin treatment. Although the regulatory mechanism by which the glutathione level is maintained is not fully understood, this discovery highlights an important new connection between cysteine and glutathione metabolism.

A logical question that emerges is how *CARS* knockdown increases the cysteine pool. *CARS* knockdown activates *de novo* cysteine synthesis via upregulation of the transsulfuration pathway (Fig. 1). In fact, not only *CARS* knockdown, but also

CONTACT Brent R. Stockwell bstockwell@columbia.edu; Kenichi Shimada ks2474@columbia.edu

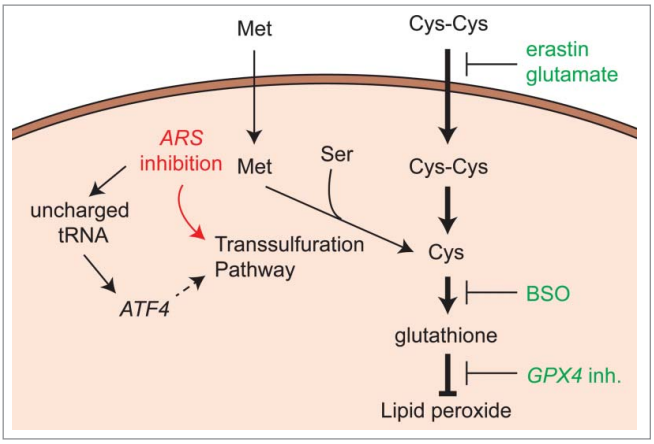

Figure 1. Inhibition of tRNA synthetases restores glutathione from the effects of cystine deprivation in ferroptosis. Ferroptosis-susceptible cells rely on cystine uptake as the primary source of cysteine. However, tRNA synthetase (ARS) inhibition can activate the transsulfuration pathway to synthesize cysteine. The dashed arrow indicates the hypothetical mechanism by which ATF4 activates the transsulfuration pathway through ARS inhibition. Compounds in green indicate ferroptosis inducers targeting different points. *ARS*, tRNA synthetase; *ATF4*, activating transcription factor 4; BSO, buthionine sulfoximine; Cys-Cys, cystine; *GPX4*, glutathione peroxidase 4; Met, methionine; Ser, serine.

inhibition of some other tRNA synthetases (ARS), including histidyl-tRNA synthetases (HARS) or glutamyl prolyl-tRNA synthetases (EPRS), were also shown to activate the transsulfuration pathway and rescue cells from erastin-induced ferroptosis; transcriptional expression of cystathionine-β-synthase (CBS), a rate-limiting enzyme of the pathway,[8] was significantly upregulated in response to knockdown of these genes. The suppressive effect of their knockdown on ferroptosis disappeared when the transsulfuration pathway was pharmacologically or genetically inhibited, confirming that *ARS* knockdown activates the transsulfuration pathway as a mechanism for preventing ferroptosis.

This study raises a number of intriguing questions. First, why does knockdown of some, but not all, ARS activate cysteine synthesis instead of the corresponding amino acids relevant to each synthase? We suspect that ARS suppression that inhibits ferroptosis activates the transcription factor activating transcription factor 4 (ATF4) (Fig. 1), which is activated in response to amino acid deprivation and uncharged tRNAs.[9] ARS inhibition increases the presence of uncharged tRNAs, which is a signal for amino acid scarcity, and activates ATF4 expression. ATF4 alters amino acid metabolism pathways, and the transsulfuration pathway is likely one of them.

Second, why is activation of the transsulfuration pathway not the general response to cystine deprivation in all cells? Some cells rely on the transsulfuration pathway as a major supply of cysteine.[10] *De novo* cysteine synthesis induced by *CARS* knockdown is not sufficient to fully suppress the consequences of glutathione deprivation. Methionine, an essential amino acid and the sole source of sulfur for cysteine biosynthesis, is needed for other biochemical reactions such as methylation; redistributing the metabolic flux of methionine may involve expensive rewiring of metabolic networks. Nonetheless, the finding that loss of CARS suppresses ferroptosis has revealed an unexpected connection between the pathways governing protein synthesis, metabolism and cell death, providing insight into how cells cope with stresses to homeostatic networks.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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