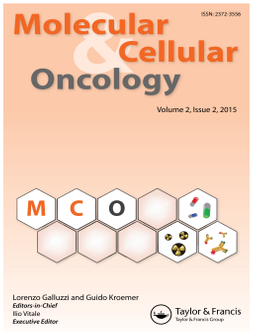

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tRNA synthase suppression activates *de novo* cysteine synthesis to compensate for cystine and glutathione deprivation during ferroptosis

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Abstract

Glutathione is a major endogenous reducing agent in cells, and cysteine is a limiting factor of glutathione synthesis. Cysteine is obtained by its uptake or biosynthesis, and mammalian cells often rely on one or the other pathway. Blockade of its uptake causes oxidative cell death due to the scarcity of glutathione, 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.

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, protecting cells from oxidative stress. Inhibition of glutathione synthesis depletes the glutathione pool in the 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 three precursor amino acids, cysteine contributes primarily to the cofactor's reduction potential—its thiol group acts as an electron donor. Glutathione-dependent oxidoredutases, such as glutathione peroxidases (*GPXs*), transfer two electrons from glutathione to effect substrate reduction; moreover, cysteine is a limiting factor for glutathione biosynthesis. Decrease in cysteine abundance leads to glutathione depletion.

Cysteine can be obtained in mammalian cells in two ways. First, mammalian 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 two amino acids, methionine and serine. This latter process is 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, as well as subsequent cell death through a regulated, non-apoptotic form of cell death termed ferroptosis[4,5]. This is the lethal mechanism that ensues after pharmacological 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, through genome-wide siRNA screening for suppressors of ferroptosis, it was discovered that knockdown of the cysteinyl-tRNA synthetase, encoded by the *CARS* gene, inhibits erastin-induced ferroptosis in multiple human and rat cell lines[7]. While erastin or glutamate-induced lethality is 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 interferes with cystine-deprivation-induced ferroptosis by erastin and glutamate. In fact, metabolomic profiling revealed that *CARS* knockdown increases cysteine levels in cells. Although glutathione abundance itself does not change upon *CARS* knockdown, 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 of how glutathione level is maintained is not fully understood, this discovery highlights an important new connection between cysteine and glutathione metabolism.

A logical question emerging 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 inhibition of some other tRNA synthetases (*ARS*), including histidyl- (*HARS*) or glutamyl-prolyl- (*EPRS*) tRNA synthetases, were also shown to activate the transsulfuration pathway and rescue cells from erastin-induced ferroptosis; transcriptional expression of cystathionine-□-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, which confirms 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). *ATF4* 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.

Secondly, 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 a 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.

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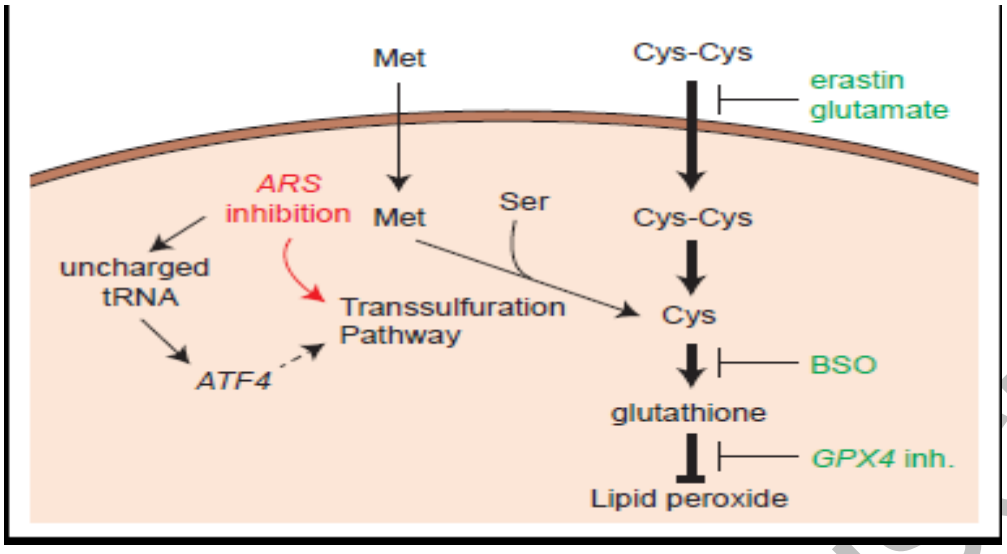

Fig. 1 Inhibition of tRNA synthetases restores glutathione from cystine deprivation in ferroptosis. Ferroptosis-susceptible cells rely on cystine uptake as the primary source of cysteine. However, tRNA synthetases (*ARS*) inhibition can activate the transsulfuration pathway to synthesize cysteine. The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