Journal of Molecular Cell Biology (2022), 14(4), mjac024 https://doi.org/10.1093/jmcb/mjac024
Published online April 18, 2022

Research Advance

Malic enzyme 2 as a therapeutic target for cancer: *comments on 'Malic enzyme 2 maintains protein stability of mutant p53 through 2-hydroxyglutarate'*

Zhenxi Zhang and Wenjing Du*

State Key Laboratory of Medical Molecular Biology, Department of Cell Biology, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences, School of Basic Medicine Peking Union Medical College, Beijing 100005, China
* Correspondence to: Wenjing Du, E-mail: wenjingdu@ibms.pumc.edu.cn

In mammalian cells, there are three isoforms of malic enzymes (MEs): a cytosolic nicotinamide adenine dinucleotide phosphate$^+$ ($NADP^+$)-dependent isoform (ME1), a mitochondrial $NAD(P)^+$-dependent isoform (ME2), and a mitochondrial $NADP^+$-dependent isoform (ME3), of which ME1 and ME2 are the major isoforms. As essential modulators of metabolism and aging, MEs have been reported to be targets for wild-type p53 (wtp53), which can transcriptionally repress their expression. Reciprocally, downregulation of ME1 and ME2 leads to wtp53 activation via distinct mechanisms (Jiang et al., 2013; Figure 1A). The TP53 gene is the most frequently mutated gene in tumors, with more than half of human cancers carrying mutations in this gene (Vogelstein et al., 2000). Compared with wtp53, the protein stability of mutant p53 (mutp53) is much higher in many tumor cells, and high levels of mutp53 usually acquire an increased oncogenic capacity. Thus, it is crucial to determine the mechanisms by which mutp53 stabilization/degradation is regulated, helping to identify therapeutic strategies that destabilize mutp53 without altering wtp53 levels. In wtp53 cells, various posttranslational modifications, including acetylation, phosphorylation, methylation, sumoylation, and ubiquitination, interfere with its proteasome-dependent clearance, resulting in p53 protein stabilization (Levine, 2019). Mutp53 is also dependent on the proteasomal pathway for degradation, and in many types of tumor cells, the accumulation of mutp53 is largely attributed to the escape from the proteasome pathway (Zhang et al., 2020). Recent studies have shown that mutp53 is regulated by different stress signals, including proteotoxic stress, DNA damage, oxidative stress, and nutrient limitation, contributing to the stabilization and accumulation of mutp53 (Mantovani et al., 2019). For example, the mevalonate pathway controls mutp53 levels through geranylgeranyl pyrophosphate (Ingallina et al., 2018). However, the mechanisms by which mutp53 escapes proteasome-dependent clearance in cancer cells are not fully understood.

In a recent publication (Zhao et al., 2022), we reported a novel function of ME2 in regulating the protein stability of mutp53 by the oncometabolite 2-hydroxyglutarate (2-HG) (Figure 1B). In cancer cells, ME2 promotes the generation of 2-HG not only by adjusting glutaminolysis but also through a catalytic reaction that requires pyruvate and reduced NADP (NADPH). Deletion of ME2 reduces the level of intracellular 2-HG, while elevated ME2 expression increases 2-HG production. Furthermore, we have identified that 2-HG directly binds to mutp53 and inhibits its ubiquitination and degradation mediated by murine double minute 2 (MDM2). Notably, addition of 2-HG can restore mutp53 protein levels and the tumor growth of ME2-deficient cells but not of the mutp53-deficient tumor cells. This study reveals a previously unappreciated catalytic function of ME2 and elucidates the mechanism by which 2-HG stabilizes mutp53 protein.

p53 binds to the N-terminal region and core domain of MDM2 through its C-terminal region, which has MDM2 ubiquitination-modified lysine residues (Poyurovsky et al., 2010). The interaction between MDM2 and p53 is repressed when the C-terminus of p53 is deleted, mutated, or acetylated. Our findings in this study reveal that 2-HG production increases the stability of mutp53 protein, but has no effect on mutp53 mRNA levels. Further mechanistic investigations demonstrate that 2-HG may bind to the C-terminal region of mutp53, but not wtp53. In addition, AutoDock Vina predicts that mutp53 residue arginine 306 (R306) is hydrogen bonded to 2-HG. Mutation of R306 to alanine (R306A) strongly reduces the binding affinity of p53(G266E) for 2-HG. Furthermore, the binding of 2-HG to mutp53 disrupts the binding of

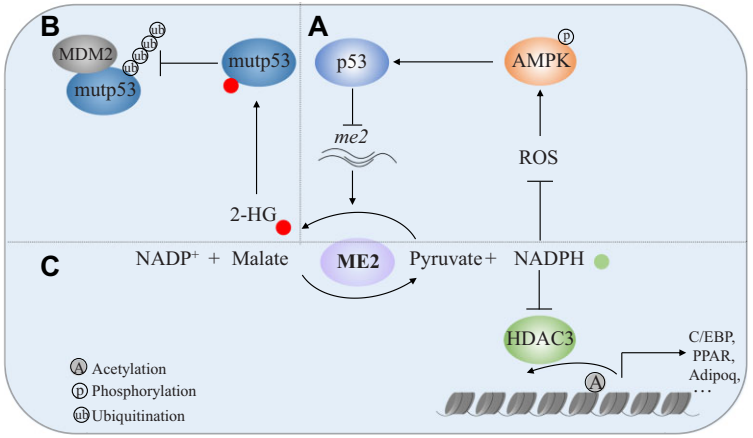

Figure 1 A diagram of the cellular functions of ME2. ME2 catalyzes the oxidative decarboxylation of malate to generate pyruvate and NADPH. (**A**) p53 transcriptionally represses ME2 expression and NADPH production. Through producing NADPH, ME2 reduces cellular reactive oxygen species (ROS) levels, thereby blocking adenosine 5′-monophosphate-activated protein kinase (AMPK)-mediated phosphorylation and activation of wild-type p53. (**B**) ME2 promotes the generation of 2-HG by adjusting glutaminolysis and through a catalytic reaction that requires pyruvate and NADPH. 2-HG directly binds to mutp53 and abolishes MDM2-mediated ubiquitination and degradation of mutp53. (**C**) As a major source of cellular NADPH, ME2 has a role in regulating chromatin remodeling and gene transcription through NADPH-mediated histone deacetylase 3 (HDAC3) inhibition.

MDM2 to mutp53, thereby reducing the ubiquitination and degradation of mutp53 by MDM2. Interestingly, the C-terminus of p53 is thought to be able to manipulate structural changes in the DNA-binding domain and determine the sensitivity of p53 to MDM2-mediated degradation (Laptenko et al., 2015). Based on these findings, it is likely that the C-terminal conformational change caused by the mutation or certain modifications may result in the selective binding of 2-HG to mutp53. Although further structural studies may be required to fully understand how 2-HG acts on mutp53, our findings reveal a role for 2-HG in directly regulating the stability of mutp53. Moreover, this work broadens the understanding of metabolic regulation of mutp53 protein degradation and provides new insights into the treatment of patients with mutp53 tumors.

The ME metabolic pathway is a major source of cellular NADPH. Our previous work identified a role for MEs (ME1 or ME2) in regulating chromatin remodeling and gene transcription through NADPH-mediated HDAC3 inhibition (Li et al., 2021; Figure 1C). As the major ME isoform in mitochondria, ME2 has the highest abundance and enzymatic activity in this organelle and participates in the tricarboxylic acid cycle and glutamine metabolism. In line with this, by adjusting glutaminolysis, ME2 can modulate the 2-HG production from glutamine (Zhao et al., 2022). 2-HG is an oncometabolite generated by reducing the ketone group of α-ketoglutarate to a hydroxyl group. In previous reports, the accumulation of 2-HG was dependent on the inhibition of its degrading enzymes (D2HGDH or L2HGDH) and the induction of mutant isocitrate dehydrogenase 1/2 or acidic pH. Our findings report ME2 as a new contributor for the intracellular 2-HG and reveal an unconventional catalytic function of ME2 in producing 2-HG by using pyruvate and NADPH. However, due to the limited sensitivity of mass spectrometry, we are currently unable to accurately determine how much 2-HG is produced from pyruvate by ME2. Nevertheless, given that significantly reduced levels of 2-HG were found in ME2-depleted tumor cells as well as in ME2-deficient mice, ME2 seems to be important for maintaining intracellular 2-HG levels.

ME2 is highly upregulated in certain types of tumors and is positively associated with tumor progression. In The Cancer Genome Atlas liver hepatocellular carcinoma cohort, elevated ME2 expression in patients with mutp53 is significantly associated with reduced patient survival, whereas ME2 expression in patients with wtp53 does not correlate with survival. Notably, in combination with previous findings that downregulation of ME2 activates wtp53 (Jiang et al., 2013), inhibition of ME2 may suppress the growth of tumors carrying either wtp53 or mutp53. Therefore, ME2 can be a potential target for tumor therapy.

[W.D. was funded by CAMS Innovation Fund for Medical Sciences (CIFMS) (2021-I2M-1-016), the National Natural Science Foundation of China (81672766), the National Key Research and Development Program of China (2019YFA0802600), and CAMS Basic Research Fund (2019-RC-HL-007).]

References

Ingallina, E., Sorrentino, G., Bertolio, R., et al. (2018). Mechanical cues control mutant p53 stability through a mevalonate–RhoA axis. Nat. Cell Biol. *20*, 28–35.

Jiang, P., Du, W., Mancuso, A., et al. (2013). Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. Nature *493*, 689–693.

Laptenko, O., Shiff, I., Freed-Pastor, W., et al. (2015). The p53 C terminus controls site-specific DNA binding and promotes structural changes within the central DNA binding domain. Mol. Cell *57*, 1034–1046.

Levine, A.J. (2019). The many faces of p53: something for everyone. J. Mol. Cell Biol. *11*, 524–530.

Li, W., Kou, J., Qin, J., et al. (2021). NADPH levels affect cellular epigenetic state by inhibiting HDAC3–Ncor complex. Nat. Metab. *3*, 75–89.

Mantovani, F., Collavin, L., and Del Sal, G. (2019). Mutant p53 as a guardian of the cancer cell. Cell Death Differ. *26*, 199–212.

Poyurovsky, M.V., Katz, C., Laptenko, O., et al. (2010). The C terminus of p53 binds the N-terminal domain of MDM2. Nat. Struct. Mol. Biol. *17*, 982–989.

Vogelstein, B., Lane, D., and Levine, A.J. (2000). Surfing the p53 network. Nature *408*, 307–310.

Zhang, C., Liu, J., Xu, D., et al. (2020). Gain-of-function mutant p53 in cancer progression and therapy. J. Mol. Cell Biol. *12*, 674–687.

Zhao, M., Yao, P., Mao, Y., et al. (2022). Malic enzyme 2 maintains protein stability of mutant p53 through 2-hydroxyglutarate. Nat. Metab. *4*, 225–238.