PNAS LETTER

Reply to Mishima et al.: MALT1 modulates GPX4 expression by regulating its stability

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We have recently published that MALT1 and the ubiquitin ligase RC3H1 cooperate to control the protein abundance of the ferroptosis regulator GPX4. In this study, MALT1 was identified as a major regulator of GPX4 in a genome-wide CRISPR screen using GPX4 protein abundance as a readout (1). In their letter to the editor, Mishima et al. argue that the MALT1 inhibitor MI-2 induces ferroptosis through direct inhibition of GPX4 (2). It is indeed correct that the MALT1 inhibitor MI-2 also inhibits GPX4, as we have published in 2023 (3). However, in our current publication, we used a genetic approach to uncover the effect of MALT1 on GPX4 protein stability. We only used MI-2 to confirm chemically that MALT1 affects GPX4 protein abundance. That MI-2 acts in this validation experiment entirely through direct inhibition of GPX4 is unlikely because we obtained an identical result on GPX4 regulation using a structurally completely unrelated small-molecule inhibitor of MALT1, safimaltib (supplemental figure 4 of ref. 1).

We do not disagree with Mishima et al. that MI-2 can cause ferroptosis through direct inhibition of GPX4. In fact, that was the message of our earlier publication (3). The disagreement is that Msihima et al. did not see that genetic ablation of MALT1 results in a decrease in GPX4 protein that we observed in multiple liver cancer cell lines. While we do not have a definitive explanation for this discrepancy, we note that Mishima et al used the two cell lines that in our experiments are the most sensitive to inhibition of GPX4 (Huh7 and SK-Hep1, see figure 3*D* of ref. 1). Because of the exquisite sensitivity of these cell lines to induction of ferroptosis, we were unable to generate stable CRISPR knockout clones for MALT1 in either Huh7 or SK-Hep1 cells. Given that MI-2 has a dual effect on both MALT1 and on GPX4, it is possible that both activities contribute to the toxicity of the drug to liver cancer cells. However, clean genetic ablation of MALT1, as used in our recent publication (1), allows us to separate these two effects and demonstrate that MALT1 protein deficiency reduces GPX4 protein abundance.

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The authors declare no competing interest.

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1. J. Wang *et al.*, Deciphering the role of the MALT1-RC3H1 axis in regulating GPX4 protein stability. *Proc. Natl. Acad. Sci. U.S.A.* **122**, e2419625121 (2025).
2. E. Mishima *et al.*, MALT1 inhibitor MI-2 induces ferroptosis by direct targeting of GPX4. *Proc. Natl. Acad. Sci. U.S.A.* **122**, e2507997122 (2025).
3. J. Wang *et al.*, Identification of a novel ferroptosis inducer with dual modulatory effects on GPX4 activity and stability. bioRxiv [Preprint] (2023). https://doi.org/10.1101/2023.12.22.572948 (Accessed 14 April 2025).