Exploring the role of GPX4 in ferroptosis induction: implications for cancer therapy

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CRISPR/Cas9 genome editing, with its precision and efficiency, holds promise for cancer treatment, potentially overcoming resistance to traditional chemotherapy via ferroptosis regulation. The study aimed to confirm the hypothesis that glutathione peroxidase 4 (*GPX4*) plays a crucial role in protecting cells from ferroptotic cell death. Research involved creating cell models - mutant lines with the *GPX4* gene knocked out, inducing ferroptosis using erastin, and analyzing the obtained data. Mutant cell lines (*GPX4* KO) were created using CRISPR/Cas9 in HCT116 WT cells. Western Blot and sequencing validated *GPX4*-deficient mutants (lines 10 and 11) with mutant 64 as positive control. Ferroptosis induction using erastin at IC_{50}-derived doses characterized cell lines for ferroptotic death. MTS viability assay showed increased susceptibility of *GPX4* KO mutant line to 10 μM erastin, unlike control lines with intact *GPX4*. Erastin reduced total glutathione levels in all cells, inversely correlating with dosage. Microscopic observations revealed a significant increase in reduced lipids positively correlating with the dose of erastin, indicating activation of an antioxidative system other than the glutathione shield. High expression of *FSP1* and *PRDX1* genes in *GPX4* KO cells indicated their ferroptosis-suppressing function. Expression of *TFRC, ACSL4, TRX*, and *PROM1* genes increased in *GPX4* KO cells, suggesting potential therapeutic targets. The results of gene expression have been previously published in the article cited below [1]. This study focused on the crucial role of *GPX4* in protecting cells from ferroptotic cell death, manifested as lipids oxidation. Analysis of lipid peroxidation and microscopic images revealed activation of an alternative antioxidative system, supporting *GPX4* KO cells. Research was supported by grant 02/040/BK_24/1056 from Silesian University of Technology.

[1] Adamiec-Organisciok M et al. (2023) Pharmaceuticals 16(12):1710

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