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Proferroptotic response to nutrient deprivation in hepatocellular carcinoma cells is related to p53 status

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Recently, it has been suggested that nutrient deprivation (ND) may be effective as an adjuvant therapy to hepatocellular carcinoma (HCC) cell treatment with sorafenib (Sfb)1. These results suggest that ND-mediated priming of HCC cells to Sfb is positively correlated with the p53 status, suggesting the essential role of p53 in priming of HCC cells for regulated cell death (RCD). Preliminary data indicated morphological signs of ferroptotic RCD, so we aimed to determine whether ferroptosis plays a role in the removal of HCC cells *in vitro* with respect to their p53 status. To this end, p53 wild-type (p53WT) and p53 knockout (p53KO) HepG2 cells were grown in growth medium or in starvation medium and treated with Sfb or with ferroptosis inducer, Rsl-3, for 6 h. Morphological signs of RCD and nuclear translocation (i.e. activation) of Nrf2, (master regulator of ferroptosis-related signalling pathways), as well as protein levels of antioxidative defence (AD) enzymes (CAT, CuZnSOD, MnSOD) and ferroptosis-related proteins (GPX4, xCT) were analysed. The AD response to Rsl-3 treatment in p53WT cells was similar regardless of nutritional status, as the level of all analysed enzymes increased. The response to Sfb was enhanced by ND as CAT and CuZnSOD were elevated. p53KO cells responded quite differently, even when treated with Rsl-3, increasing only MnSOD. Starved Sfb-treated p53KO cells even decreased expression of AD enzymes. All signs of a proferroptotic response examined were present in starved p53WT cells (regardless of treatment): decreased nuclear translocation of Nrf2, GPX4, and xCT expression. Nrf2 activation and GPX4 expression were also decreased in starved p53KO cells (especially upon treatment with Sfb or Rsl-3), but accompanied by compensatory overexpressed xCT. These results may be indicative of enhanced AD in p53KO cells and may therefore explain, at least in part, their resistance to treatment with Sfb+ND which, as presented here, induces ferroptosis in p53WT HepG2 cells.

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