Targeting Heme Oxygenase in Ferroptosis: a Novel Insight in Cancer Therapy

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1 Background

Ferroptosis is a recently identified type of programmed cell death (PCD), and it was first described by Dixon et al. in 2012 [1]. Ferroptosis is mainly characterized by iron accumulation, which leads to greatly increased ROS production and lipid peroxidation. Several studies have demonstrated the high potential of ferroptosis as a novel therapeutic strategy for cancer treatment. Among the regulators of the ferroptotic process, nuclear factor erythroid 2-related factor (Nrf2) has been gaining a great deal of attention as a multifunctional regulator of cellular redox balance and protective antioxidant response systems, including heme oxygenase (HO) enzymes [2]. Recent findings seem to propose a different approach for pharmacological modulation of HO-1 in cancer therapy, displaying a reduction in cancerous cell proliferation following enzyme induction [3]. Hence, the emerging correlation between modulation of the HO system and ferroptosis can be considered as a potential new path to pursue for treating specific cancers.

3 Conclusions

Ferroptosis has emerged as promising alternative to conventional cancer treatments. Our study focused on the modulation of one of the possible factors implicated in ferroptosis, the HO system. Our results confirmed that enzyme induction in this context can be useful to accelerate ferroptotic process. Furthermore, it was interesting to notice that HO-2, the constitutive isoform of the enzyme whose role in cancer is still unclear, seems to be involved in the erastin-triggered process. In conclusion, our data confirm that HO can be considered as a modulator factor in ferroptosis.

REFERENCES

[1] Dixon, S.J. et al. **Ferroptosis: An iron-dependent form of nonapoptotic cell death**. *Cell* 2012, 149.

[2] Xiang, M. et al. **Nrf2: Bane or blessing in cancer?** *J. Cancer Res. Clin. Oncol.* 2014, 140.

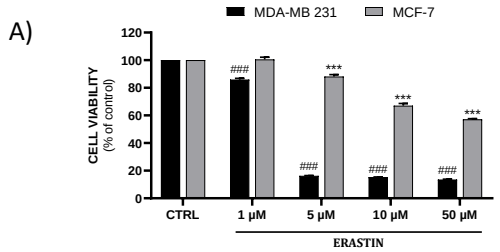
[3] Tertil, M. et al. **Nrf2-heme oxygenase-1 , axis in mucoepidermoid carcinoma of the lung: Antitumoral effects associated with down-regulation of matrix metalloproteinases**. *Free Radic. Biol. Med.* 2015, 89.

[4] Cao, X. et al. **Curcumin suppresses tumorigenesis by ferroptosis in breast cancer**. *PLoS ONE* 2022, 17.

[5] Salerno, L. et al. **Novel imidazole derivatives as heme oxygenase-1 (HO-1) and heme oxygenase-2 (HO-2) inhibitors and their cytotoxic activity in human-derived cancer cell lines.** *Eur. J. Med. Chem.* 2015, 96.

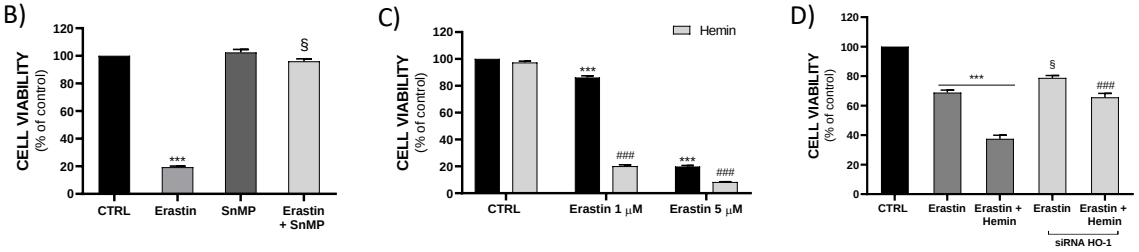
2 Results

Breast cancer cells sensitivity to ferroptosis



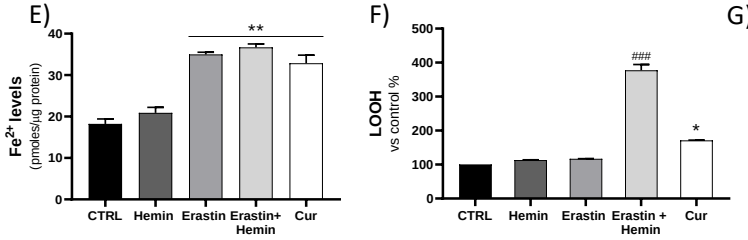
Ferroptosis model was obtained in MDA-MB 231 and MCF-7 breast cancer cell lines using ferroptosis inducer erastin at different concentrations **(A)**. The response to erastin was significantly different in the two breast cancer cell lines; notably, cell viability was greatly decreased after 48 h of treatment in MDA-MB 231 compared with MCF-7. (###p<0.0005 vs untreated MDA-MB 231; ***p<0.0005 vs untreated MCF-7)

HO-1 modulation regulates ferroptosis in triple negative breast cancer cells



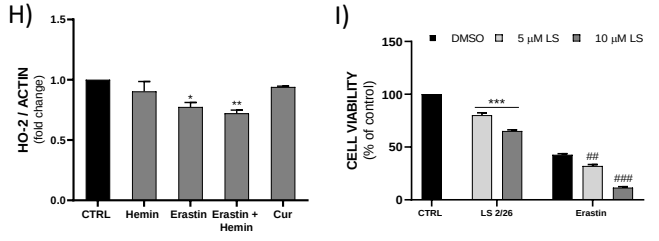
Cells were treated with non-toxic concentrations of a well-known HO inhibitor (SnMP) and its enzymatic substrate (hemin). Co-treatment with SnMP and erastin surprisingly reversed erastin's cytotoxic effect **(B)** (***p<0.0005 vs CTRL; § p<0.0005 vs erastin), while co-treatment with hemin markedly potentiated it **(C)** (***p<0.0005 vs CTRL; ###p<0.0005 vs erastin). To better understand and elucidate HO-1 involvement in this process, we decided to use HO-1 siRNA; although erastin's cytotoxic effect on HO-1-silenced cells was slightly but significantly reduced, co-treatment with hemin was considerably less effective compared with HO-1-expressing cells **(D)** (***p<0.0005 vs CTRL; § p<0.0005 vs erastin; ###p<0.0005 vs erastin + hemin).

HO-1 induction alters redox homeostasis leading to ferroptosis



In order to investigate HO-1 induction impact in the ferroptotic mechanism we evaluated Fe^{2+} accumulation and lipid hydroperoxides levels following treatment with combination of erastin and HO substrate hemin, and curcumin (Cur), which is known to be a natural HO-1 inducer and has been associated to ferroptosis induction in breast cancer cells [4]. Fe^{2+} and lipid hydroperoxide (LOOH) levels were elevated after 24 h of treatment **(E,F)** (*p<0.05, **p<0.005 vs CTRL; ##p<0.005, ###p<0.0005 vs erastin); in particular Fe^{2+} levels were similar for the erastin and both curcumin/co-treatment groups, while LOOH levels were significantly increased only after the erastin-hemin combination treatment. Hence, inducing HO-1 may alter redox homeostasis, leading to lipid peroxidation. in accordance with LOOH levels, BODIPY results showed increased lipid ROS production especially after combination treatment **(G)** (*p<0.05 vs CTRL; ##p<0.005, ###p<0.0005 vs erastin).

HO-2 involvement in ferroptosis



Western blot analysis was performed for HO constitutive isoform HO-2. Surprisingly, we observed a significant reduction in HO-2 protein levels in the erastin and erastin/hemin combination groups **(H)** (*p<0.05, **p<0.005 vs CTRL), suggesting the possible implication of HO-2 in erastin-induced ferroptosis. Subsequently, we tested an HO-2-selective inhibitor, LS 2/26 [5], to investigate HO-2 role in the ferroptotic process. LS 2/26 showed a synergistic effect when combined with erastin, potentiating its cytotoxicity **(I)** (***p<0.0005 vs CTRL; ##p<0.005, ###p<0.0005 vs LS 2/26) .