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Article

Elevated GPx4 and FSP1 expression in MG63 cells: potential links to drug resistance and ferroptosis

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SUMMARY

Osteosarcoma (OS) treatments currently rely on a combination of surgery and chemotherapy. However, the development of drug resistance remains a major obstacle, significantly impacting the five-year survival rate of OS patients. Addressing this issue requires new therapeutic approaches. Ferroptosis, a type of regulated cell death driven by oxidative stress, has emerged as a potential mechanism for cancer treatment. Ferroptosis is distinct from other forms of cell death and has been explored in various cancers, but its role in OS remains under investigation. We hypothesized that the antioxidant enzymes GPx4 and FSP1 are expressed at significantly higher levels in OS cells, particularly in MG63 cells, compared to healthy osteoblasts. This elevated expression suggests a potential role in inhibiting ferroptosis. To explore this, we investigated the differential expression of GPx4 and FSP1 in MG63 cells and healthy osteoblasts to understand their relationship to ferroptosis inhibition. Our results support this hypothesis, showing that higher levels of GPx4 and FSP1 in MG63 cells correlate with inhibition of the ferroptosis pathway. This suggests that the accumulation of these proteins plays a role in blocking ferroptosis, potentially contributing to drug resistance in OS. These findings open the door to new therapeutic strategies aimed at overcoming drug resistance by targeting the pathways that regulate ferroptosis. By manipulating these mechanisms, we may be able to enhance the effectiveness of chemotherapy and improve patient outcomes.

INTRODUCTION

Osteosarcoma (OS) is the most common primary malignant bone cancer, primarily affecting adolescents and young adults. Despite advances in treatment, the five-year relative survival rate for OS remains around 70% with limited improvement over the past several decades(1). The survival rate for patients with metastases is particularly poor, underscoring the need for more effective therapies. Therefore, improving the efficiency of surgery and chemotherapy to raise the survival rate of OS patients is a major goal for current research (2).

There are two major problems that affect the effectiveness of current treatments for OS. The first is drug resistance, which occurs genetically or environmentally, and the second is the development of metastasis. Drug resistance develops based on several factors. Various DNA repair pathways such as nucleotide excision repair and base excision repair have been linked to drug resistance (3). While this kind of resistance mainly works on drugs like cisplatin, ifosfamide, and doxorubicin, methotrexate resistance presents a unique scenario (3). The primary mechanism of methotrexate is binding with dihydrofolate reductase (DHFR), leading to an inhibition of DNA synthesis and replication, which subsequently causes apoptosis. However, overaccumulation of DHFR can cause resistance to methotrexate. Resistance occurs through a decrease in the expression of the membrane-located solute carrier family 19 member 1(SLC19A1), thus harming the transportation pathway followed by an increase in the expression in DHFR (3,4). In recent studies, biomarkers such as ATP binding cassette (ABC) subfamily B membrane 1(ABCB1) and excision repair cross-complementation group 1 (ERCC1) were discovered to affect drug sensitivity in different ways. ABCB1 is a membrane transporter protein that functions as a “drug pump,” actively expelling drugs from the cell. This reduces the drug’s intracellular concentration, preventing it from reaching a effective level (5). ERCC1 facilitates efficient DNA repair, allowing cancer cells to repair DNA damage caused by platinum drugs and thus reducing the effectiveness of these drugs (6). Other factors such as extracellular vesicles and non-coding RNAs can also affect drug sensitivity and contribute to drug resistance (3).

In 2012, a new form of cell death was discovered, ferroptosis, which has been found to play a role in drug resistance (7). Ferroptosis is an iron-dependent form of programmed cell death, marked by the buildup of lipid peroxides (8). It differs genetically and biochemically from other regulated cell death types (8). Ferroptosis begins when glutathione-dependent antioxidant defenses fail, leading to an uncontrolled increase in lipid peroxides and, ultimately, cell death (8). Studies have revealed the role of this newly discovered pathway in drug resistance in various type of cancers (9-13). Specifically, failure of ferroptosis through resistance mechanisms can play a crucial role in increasing chemotherapeutic drug resistance.

The mechanism of ferroptosis can be inhibited by two primary antioxidant systems: glutathione peroxidase 4 (GPx4), which catalyzes the reduction of lipid peroxides through a glutathione-dependent reaction, and Ferroptosis suppressor protein 1 (FSP1), which aids in regenerating ubiquinone or coenzyme Q10 (CoQ10), and acts as a lipid peroxyl radical scavenger (14). GPx4 reduces lipid peroxides, thereby preventing the accumulation of lethal reactive oxygen species (ROS), while FSP1 promotes ferroptosis resistance by recycling CoQ10 to neutralize lipid peroxidation (15). In

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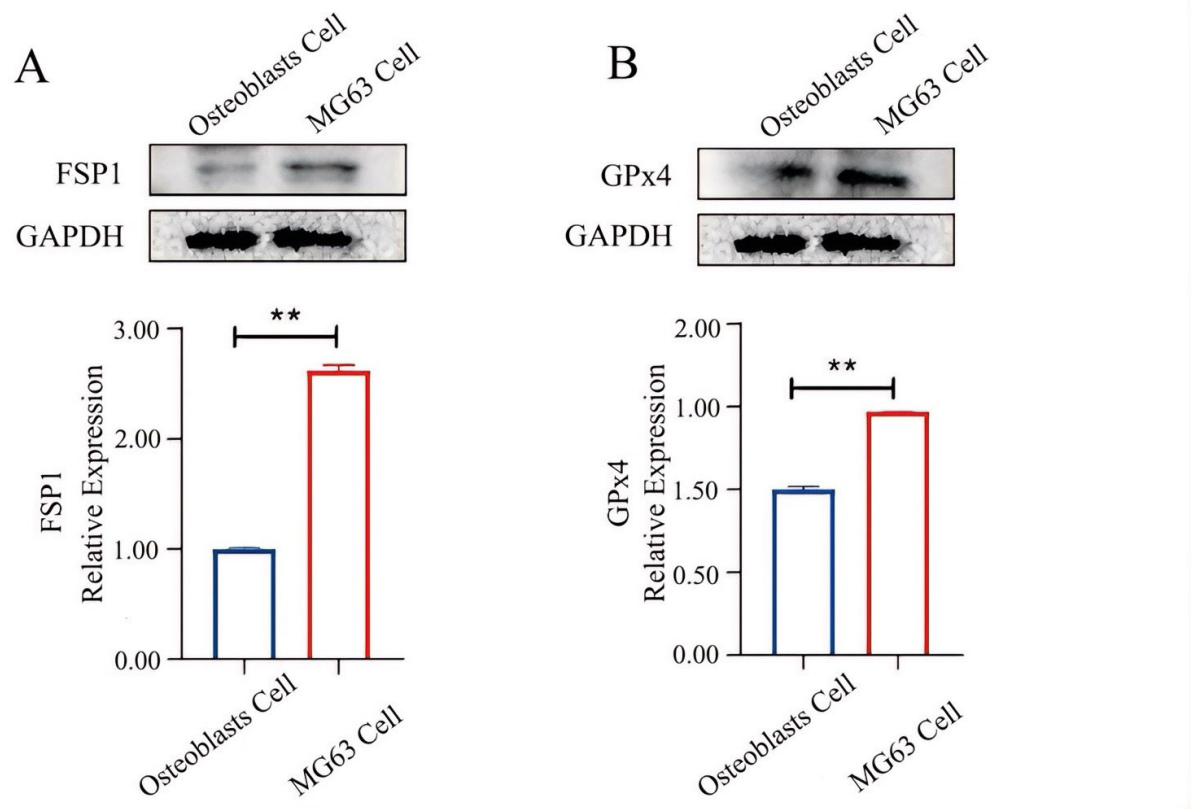

Figure 1: Western blot and quantified relative expression of the GPx4 and FSP1 in MG63 cells and osteoblast cells. A) Relative FSP1 expression in MG63 OS cells and osteoblasts normalized by GAPDH. B) Relative GPx4 expression in MG63 OS cells and osteoblasts normalized by GAPDH. t-test, **p<0.01, n = 4

order to survive in harsh environments, cancer cells need to increase their antioxidant defense capacity to enhance their ability to survive under oxidative stress. New drugs and treatments target the antioxidant defenses of cancer cells, aiming to make cancer cells more vulnerable to oxidative stress-induced cell death, including apoptosis and ferroptosis, thereby promoting cancer cell death (16).

We hypothesized that OS cells, specifically MG63 cells, are able to resist ferroptosis through the accumulation of GPx4 and FSP1. To test this hypothesis, our research focused on measuring the difference in the expression levels of these proteins between MG63 cells and healthy osteoblasts. By examining the expression of key ferroptosis inhibitors GPx4 and FSP1, we found evidence suggersting that ferroptosis may be suppressed in osteosarcoma cells, highlighting a potential resistance mechanism that could inform future therapeutic strategies. By understanding the relationship between ferroptosis and OS, we can further explore and overcome the drug resistance of OS through ferroptosis, thereby improving the survival rate of patients.

RESULTS

To investigate whether ferroptosis occurs in MG63 cells, we measured the expression levels of GPx4 and FSP1, and compared them to that of healthy osteoblasts. We analyzed these factors to determine if GPx4 and FSP1 inhibit ferroptosis in MG63 cells.

Using Western blot our results show that FSP1 protein levels were significantly higher in MG63 cells than in healthy osteoblasts (t-test, p<0.01; **Figure 1A**). This finding indicates an accumulation of FSP1 protein within MG63 cells. Similarly, we found elevated levels of GPx4 protein in MG63 cells compared to osteoblasts, confirming GPx4 accumulation in these cells (t-test, p<0.01; **Figure 1B**).

The combined findings demonstrate that MG63 cells expressed GPx4 and FSP1 are at significantly higher levels than healthy osteoblasts. This differential expression suggests that GPx4 and FSP1 may play a role in inhibiting ferroptosis in MG63 cells.

DISCUSSION

This study provides an important initial step in exploring potential strategies to address drug resistance in OS treatment through the mechanism of ferroptosis. Our findings demonstrate that both GPx4 and FSP1 are expressed at significantly higher levels in MG63 OS cells compared to healthy osteoblasts Although these results do not directly confirm ferroptosis inhibition, they align with the established roles of GPx4 and FSP1 as critical regulators of ferroptosis. Their elevated expression in OS cells suggests that GPx4 and FSP1 may play a role in reducing the susceptibility of cancer cells to ferroptosis, thereby supporting their survival and potentially contributing to drug resistance.

Literature has documented that GPx4 and FSP1 function as inhibitors of ferroptosis through distinct yet complementary mechanisms (8). GPx4 reduces lipid peroxides through glutathione, thereby preventing the accumulation of lethal reactive oxygen species (ROS), while FSP1 reduces them

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through CoQ10 to neutralize lipid peroxidation (8). The higher expression of these proteins in MG63 cells indicates a robust antioxidant system that may protect these cancer cells from ferroptosis-induced cell death. This observation aligns with studies in other cancer types, where ferroptosis is a key mechanism of cell death often circumvented by cancer cells (9-13). For instance, in oral squamous cell carcinoma, ferroptosis is inhibited through NTP-catalyzed reduction of Fe^{3+} to Fe^{2+}, coupled with lipid peroxidation and mitochondrial superoxide production (15). Similarly, in breast cancer, ferroptosis resistance arises from reduced iron export due to the suppression of prominin 2 and the formation of multivesicular bodies (10). These examples highlight the diversity of mechanisms by which cancer cells evade ferroptosis, suggesting that the precise regulatory pathways in OS require further investigation.

While the elevated levels of GPx4 and FSP1 observed in this study lay the foundation for understanding ferroptosis suppression in OS, our results do not directly establish their functional roles in this process. Importantly, our study does not include experiments designed to evaluate ferroptosis activity or drug resistance directly, which are critical for confirming the proposed connections. Thus, while our findings suggest that GPx4 and FSP1 may contribute to ferroptosis inhibition in MG63 cells, further research is needed to validate these hypotheses. Functional studies, including genetic knockdown or pharmacological inhibition of GPx4 and FSP1, are needed to establish causality and determine their contribution to ferroptosis suppression and drug resistance.

Another important limitation of this study is the small sample size, which reduces the generalizability of our findings. Future studies should include larger, more diverse OS cell lines from different patients to confirm the observed expression patterns of GPx4 and FSP1 and to account for potential environmental or demographic variations. For instance, factors such as altitude, living environment, or genetic background may influence the expression or activity of these proteins. Expanding the scope of this research to include samples from diverse populations could provide a more comprehensive understanding of the role of GPx4 and FSP1 in OS.

In addition to addressing these limitations, future research should focus on elucidating the specific mechanisms by which GPx4 and FSP1 interact to inhibit ferroptosis in OS cells. It is unclear whether these proteins act independently, synergistically, or competitively in this context. Understanding their relationship could reveal new therapeutic opportunities. For example, if one protein plays a dominant role in ferroptosis suppression, targeting that protein could yield more effective treatments. Alternatively, if the proteins function collaboratively, dual inhibition strategies might be required.

From a translational perspective, this study highlights the potential for targeting ferroptosis regulators such as GPx4 and FSP1 to overcome drug resistance in OS. Therapies designed to modulate ferroptosis could enhance the efficacy of existing treatments and improve patient outcomes. Furthermore, the development of targeted therapies could reduce the side effects associated with conventional chemotherapies, improve drug sensitivity, and potentially decrease the need for radical surgical interventions such as amputation. In the long term, these strategies could contribute to improved survival rates and quality of life for OS patients.

In conclusion, this study provides a foundation for understanding the potential roles of GPx4 and FSP1 in ferroptosis suppression in OS. While our findings suggest that these proteins may contribute to drug resistance, they also underscore the need for further research to elucidate the underlying mechanisms and validate these connections. Future work should focus on functional studies, larger sample sizes, and mechanistic investigations to pave the way for novel therapeutic approaches targeting ferroptosis in OS. By addressing these gaps, we hope to contribute to the development of effective treatments that meet the urgent need to overcome drug resistance and improve outcomes for patients with this aggressive cancer.

MATERIALS AND METHODS

Cell Lines and Culture Conditions

MG63 cells (Abiowell, Part Number: AW-CCH046) were cultured up to the 10th passage in a high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin dual antibiotic. Osteoblast cells (Abiowell, Part Number: AW-CNH491) were similarly cultured up to the 10th passage using osteoblast-specific medium (Procell, CM-H111). Both cell lines were maintained at 37°C in a humidified atmosphere containing 5% CO_2 and ~95% relative humidity.

Protein Extraction and Western Blot Analysis

Protein extraction was performed when the cell density reached ~80%, roughly 2-3 days after the initial culture. Western blotting was used to quantify the expression levels of GPX4 and FSP1 proteins. Proteins were separated based on molecular weight using sodium dodecyl sulfate–polyacrylamide (SDS-PAGE) gel electrophoresis. The SDS-PAGE gel for GPx4 and GAPDH were both at 10% of polyacrylamide, and the gel for FSP1 was used at 12% of polyacrylamide. Proteins were transferred to a polyvinylidene fluoride (PVDF) membrane. Membranes were probed with primary antibodies specific to GPX4 (Affinity, DF6701), FSP1 (Proteintech, 20886-1-AP), and GAPDH (Proteintech, 60004-1-Ig) followed by horseradish peroxidase (HRP)-conjugated secondary antibodies (Proteintech, SA-00001-2 and SA00001-1) for detection (12). The chemiluminescence signal was visualized using Image Lab 6.0 software, and protein bands were quantified relative to the internal control GAPDH. T-tests were carried out during the experiment using GraphPad Prism. Data were assessed for normal distribution using the Shapiro-Wilk test. The variances were assumed to be equal. A p-value < 0.01 was considered statistically significant.

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REFERENCES

1. American Cancer Society. *Key Statistics For Osteosarcoma.* 08 Oct. 2020, https://www.cancer.org/cancer/types/osteosarcoma/about/key-statistics.html
2. Corre, Isabelle., et al "The Osteosarcoma Microenvironment: A Complex but Targetable Ecosystem." *Cells*, vol. 9, no. 4, 15 Apr. 2020, pp. 976. https://doi.org/10.3390/cells9040976
3. Hattinger CM., et al "Drug Resistance in Osteosarcoma: Emerging Biomarkers, Therapeutic Targets and Treatment Strategies." *Cancers.* vol. 13, no. 12, 9 Jun. 2021, pp. 2878. https://doi.org/10.3390/cancers13122878
4. Hashizume, M., et al. "Interleukin-6 regulates anti-arthritic effect of methotrexate via reduction of SLC19A1 expression in a mouse arthritis model." *Arthritis Res Ther* , vol. 14, no. 96 30 Apr. 2012, https://doi.org/10.1186/ar3821
5. Chen KG, Sikic BI. "Molecular pathways: regulation and therapeutic implications of multidrug resistance." *Clin Cancer Res*. vol. 18, no.7, 16 Feb. 2012 https://doi.org/10.1158/1078-0432.CCR-11-1590
6. Wu, Jiaohong, et al. "Meta-analysis of excision repair cross-complementation group 1 (ERCC1) expression and platinum chemosensitivity in patients with ovarian cancer." *APM*, vol 9, no 5, 30 Sep 2020. https://doi.org/10.21037/apm-20-1634
7. Yan, H.f., et al. "Ferroptosis: mechanisms and links with diseases." *Signal Transduction and Targeted Therapy*, vol. 6, no. 49, 03 Feb. 2021, pp. 49. https://doi.org/10.1038/s41392-020-00428-9
8. Tan, Shirlee., et al "Oxytosis: A Novel Form of Programmed Cell Death." *Current Topics in Medicinal Chemistry*, vol. 1, no. 6, 2001, pp. 497-506. http://dx.doi.org/10.2174/1568026013394741
9. Zhou, Q., et al. "Ferroptosis in cancer: from molecular mechanisms to therapeutic strategies." *Natur,* vol. **9**, no. 55 08 Mar. 2024, https://doi.org/10.1038/s41392-024-01769-5
10. Hattinger, C. M., et al. " Drug Resistance in Osteosarcoma: Emerging Biomarkers, Therapeutic Targets and Treatment Strategies." *Cancers*, vol. 13 no. 12, 09 Jun. 2021. https://doi.org/10.3390/cancers13122878
11. Li, Z., Chen, L., Chen, C. *et al.* "Targeting ferroptosis in breast cancer*".Biomark Res* vol. **8**, no. 58, 05. Nov.2020. https://doi.org/10.1186/s40364-020-00230-3
12. Zhao, Lei, et al. *"*Ferroptosis in cancer and cancer immunotherapy.*"* *Cancer Communications*, vol. 8, no.2 Feb. 2022, pp.88-116 https://doi.org/10.1002/cac2.12250
13. Tang, D., et al. "Ferroptosis: molecular mechanisms and health implications." *Cell Res*, vol **31**, 02 Dec, 2020, pp.107–125 https://doi.org/10.1038/s41422-020-00441-1
14. Dixon, S. J., et al. "Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death." *Cell*, vol. 149, no. 5, 25 May 2012, pp. 1060-1072. https://doi.org/10.1016/j.cell.2012.03.042
15. Bersuker, K., et al. "The CoQ Oxidoreductase FSP1 Acts Parallel to GPX4 to Inhibit Ferroptosis." *Nature*, vol. 575, 21Oct. 2019, pp. 688-692. https://doi.org/10.1038/s41586-019-1705-2
16. Panczyszyn, E., et al. "FSP1 is a predictive biomarker of osteosarcoma cells' susceptibility to ferroptotic cell death and a potential therapeutic target." *Cell Death Discovery*. vol. 10, no. 87, 17 Feb. 2024, pp. 87. https://doi.org/10.1038/s41420-024-01854-2