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Association of Ferroptosis-Related Genes with Iron Accumulation and Clinicopathological Features in Colorectal Cancer

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Abstract

Purpose

Ferroptosis, an iron-dependent form of regulated cell death, has emerged as a critical mechanism in cancer biology and therapeutic resistance. This study aims to investigate the clinical relevance of key ferroptosis regulators, including HO-1, GPX4, and SLC7A11, in patients with colorectal cancer (CRC).

Methods

A total of 103 CRC tissue samples were analysed. Gene expression levels of HO-1, GPX4, and SLC7A11 were assessed, and their associations with clinicopathological features were evaluated. Iron concentrations were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES). Associations between biomarker expression, iron levels, and clinicopathological characteristics were analysed.

Results

HO-1 and GPX4 expression were significantly higher in patients aged ≤ 69 years compared with older patients ($p = 0.027$ and $p = 0.008$, respectively). HO-1 expression was significantly associated with microsatellite stability ($p = 0.026$). Also, SLC7A11 expression was significantly associated with tumour site ($p = 0.037$), with higher expression in proximal colon tumours, and was also associated with tissue iron levels ($p = 0.031$). Significant associations were identified between HO-1 and GPX4 expression ($p < 0.001$) and a marginal association between HO-1 and SLC7A11 ($p = 0.062$).

Conclusion

Ferroptosis-related genes are associated with clinicopathological features and tissue iron levels in colorectal cancer. The relationships among HO-1, GPX4, and SLC7A11 suggest interactions between iron metabolism, oxidative stress, and ferroptosis pathways, highlighting potential therapeutic targets in CRC.

INTRODUCTION

Colorectal cancer (CRC) remains one of the leading causes of cancer-related morbidity and mortality worldwide, representing a major global health burden (Yeo, Hwang et al. 2026). Despite advances in surgery, chemotherapy, targeted therapy, and immunotherapy, intrinsic and acquired therapeutic resistance remain major obstacles to improving long-term survival. Consequently, there is an urgent need to identify reliable biomarkers that predict therapeutic response and uncover novel therapeutic targets (Ou, Wu et al. 2024, Mahemuti, Ma et al. 2026).

Ferroptosis, a distinct form of regulated cell death characterised by iron-dependent lipid peroxidation, has emerged as a critical mechanism in cancer biology (Dixon, Lemberg et al. 2012). Unlike apoptosis, ferroptosis is driven by iron-dependent lipid peroxidation resulting from dysregulated iron metabolism and oxidative stress (Stockwell, Angeli et al. 2017). Accumulating evidence suggests that cancer cells evade ferroptotic cell death through activation of antioxidant defence pathways, thereby facilitating tumour survival, progression, and therapeutic resistance (Jiang, Stockwell et al. 2021, Dewasi and Jain 2026). Among the principal regulators of ferroptosis, glutathione peroxidase 4 (GPX4) plays a pivotal role by detoxifying lipid hydroperoxides and preventing oxidative membrane damage (Chen 2026). Similarly, SLC7A11, a key component of the cystine/glutamate antiporter system Xc^-, maintains intracellular glutathione homeostasis and protects cells against oxidative stress-induced injury (Koppula, Zhuang et al. 2021). Increased expression of GPX4 and SLC7A11 promotes resistance to ferroptosis and has been associated with poor therapeutic response and adverse prognosis in several malignancies, including CRC (Wu, Shen et al. 2022, Ou, Wu et al. 2024).

Heme oxygenase-1 (HO-1) is an important regulator of ferroptosis (Tang, Ju et al. 2021). While moderate HO-1 activity provides cytoprotection through antioxidant mechanisms, excessive HO-1 activity may increase intracellular iron accumulation and subsequently promote ferroptosis under specific cellular conditions (Xu, Chen et al. 2026). This dual role underscores the complex

interplay between oxidative stress, iron metabolism, and ferroptotic signalling pathways in cancer pathogenesis. In CRC, dysregulation of iron metabolism has increasingly been reported in tumour initiation, progression, and therapeutic response. Elevated intracellular iron levels may enhance oxidative stress and lipid peroxidation, thereby sensitising tumour cells to ferroptosis (Ahmadi, Vidanapathirana et al. 2025). However, colon cancer cells may circumvent ferroptotic cell death through upregulation of antioxidant defence mechanisms, particularly GPX4 and SLC7A11, resulting in the development of a ferroptosis-resistant phenotype(Ou, Wu et al. 2024).

Despite this growing evidence in ferroptosis-mediated colorectal carcinogenesis, the clinical significance of ferroptosis-related genes and their interactions with iron metabolism in CRC remains poorly understood. Therefore, the present study aims to investigate the expression profiles of key ferroptosis-related genes, including HO-1, GPX4, and SLC7A11, in colorectal cancer tissues, evaluate their association with clinicopathological parameters, and explore their relationship with iron levels. Understanding these molecular interactions will offer novel insights into the regulation of ferroptosis in CRC and identify potential therapeutic targets.

MATERIAL AND METHOD

Patient Recruitment and Tissue Collection

A total of 103 patients with CRC undergoing surgical resection at Gold Coast University Hospital, Queensland, Australia, were recruited for this study. All patients were newly diagnosed with CRC and had not received prior chemotherapy or radiotherapy before surgery. Patient recruitment was performed without selection bias, and written informed consent was obtained from all participants prior to sample collection. Paired tumour and non-neoplastic tissue samples (away from the tumour and near resection margins) were collected following surgical resection. The non-neoplastic tissue was obtained from macroscopically uninvolved mucosa located at least 5 cm from the tumour margin and was confirmed histologically to be free of tumour infiltration. Fresh tumour tissues and corresponding non-neoplastic tissues were immediately snap-frozen in liquid nitrogen and stored at − 80°C until further molecular and biochemical analyses. Half of the matched tumour and non-neoplastic tissue were preserved in formalin and processed for routine histopathological examination to confirm the diagnosis.

In brief, clinicopathological characteristics, including tumour size, anatomical location, tumour perforation, histological subtype, microsatellite instability (MSI) status, lymphovascular invasion (LVI), and tumour stage, were analysed by the authors (CTL and AL). Proximal tumours included those arising in the caecum, ascending colon, and transverse colon, whereas distal tumours included tumours located in the descending colon, sigmoid colon, and rectum. Histopathological classification was performed according to the World Health Organisation (WHO) Classification of Tumours of the Digestive System, and tumour staging was determined using the American Joint Committee on Cancer (AJCC) TNM staging system (8th edition). Ethical approval for the collection and analysis of human tissue was obtained from the Griffith University Human Research Ethics Committee (GU Ref No: MSC/17/10/HREC), and written informed consent was obtained from all participants prior to inclusion in the study. All procedures involving human participants and biological specimens were conducted in accordance with the approved ethical guidelines and institutional requirements.

Quantitative Real-Time PCR (qPCR):

Total RNA was extracted from fresh-frozen colorectal tissue samples using the QIAgen RNA extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The concentration and purity of the extracted RNA were determined using a NanoDrop spectrophotometer by measuring the absorbance ratios at 260/280 nm and 260/230 nm. RNA integrity was assessed before downstream analysis. Complementary DNA (cDNA) was synthesised from 1 µg of total RNA using a reverse transcription kit according to the manufacturer's protocol. Quantitative real-time PCR (qPCR) was performed using the Luna® Universal qPCR Master Mix (New England Biolabs, Ipswich, MA, USA) on a QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

Gene expression levels of HO-1, GPX4, and SLC7A11 were quantified using gene-specific primers. Primer sequences used for amplification of HO-1, GPX4, SLC7A11, and GAPDH are provided in Table 1. Each reaction was performed in a final volume of 20 µL containing 10 µL of 2× Luna Universal qPCR Master Mix, forward and reverse primers, cDNA template, and nuclease-free water. All

reactions were run in triplicate, and no-template controls were included to assess potential contamination. The thermal cycling conditions consisted of an initial denaturation at 95°C for 60 seconds, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 30 seconds. Melt curve analysis was performed at the end of each run to verify amplification specificity. Relative gene expression levels were normalised against the housekeeping gene GAPDH and calculated using the comparative 2^-ΔΔCt method. Results were expressed as fold changes relative to the corresponding normal tissue controls(Livak and Schmittgen 2001). The fold change values were categorised as low and high expressions (≤ 2 was considered as low expression and > 2 as high expression)(Gamage, Islam et al. 2021).

Iron Quantification by ICP-OES

46 fresh tissue samples were freeze-dried for 48 hours to remove residual moisture prior to elemental analysis. The dried samples were transferred into individual Berghof DAP100 + digestion vessels and digested using 3 mL of concentrated nitric acid (~ 70%). Blank samples were prepared in parallel to monitor potential contamination during the digestion process and account for background elemental contributions from the reagents. Following sufficient outgassing, digestion vessels were securely sealed and loaded into a Berghof Speed wave Four microwave digestion system. Due to the capacity limitation of the microwave system (12 vessels per run), samples were processed in sequential batches. Microwave-assisted digestion was performed for 45 minutes with a temperature ramp reaching a maximum of 175°C, enabling efficient oxidation and complete digestion of organic tissue components under high-pressure and high-temperature conditions. After completion of the digestion cycle, vessels were allowed to cool to room temperature before the digested samples were transferred into 10 mL volumetric flasks. Each digestion vessel was rinsed thoroughly with ultrapure water, and the rinse solutions were added to the corresponding volumetric flasks to ensure complete sample recovery. The final volume was adjusted to 10 mL with ultrapure water. Iron concentrations were subsequently determined by inductively coupled plasma optical emission spectrometry ICP-OES and expressed as µg/g dry weight(Vidanapathirana, Woon et al. 2026).

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics, Version 31.0 (IBM Corp., Armonk, NY, USA). Categorical variables are presented as frequencies and percentages. Associations between gene expression status and clinicopathological characteristics were assessed using Pearson's chi-square test or Fisher's exact test, as appropriate, according to the expected cell frequencies. All statistical tests were two-sided, and a P-value of < 0.05 was considered statistically significant.

Table 1
Primer Sequence

Gene	Forward	Reverse
HO-1	CAACAAAGTGCAAGATTCTG	TGCATTCACATGGCATAAAG
GPX-4	CAGTTCGGGAAGCAGGAG	GCCCTTGGGTTGGATCTT
SLC7A11	ATGCAGTGGCAGTGACCTTT	GGCAACAAAGATCGGAACTG
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

Table 2
Patients Characteristics

Demographic characteristics	Tissue n = 103(%)
Gender	
Male	50 (48.54)
Female	53 (51.46)
Age	
≤ 69 years	58(56.3)
> 69 years	45 (43.7)
Size	
≤ 40 mm	50(48.5)
> 40 mm	53(51.5)
Site	
Colon	74(71.8)
Rectum	29(28.2)
Garde	
Well (1)	13(12.6)
Moderate (2)	80(77.6)
Poor (3)	10(9.8)
T stage	
I or II	47(45.6)
III or IV	56(54.4)
LVI	
Negative	58(56.3)
Positive	45(43.7)
Metastasis	81(78.6)
M0	22(21.4)
M1	96(93.2)
Tumour Perforation	7(6.8)
NO	84(81.6)
YES	19(18.4)
MSI status	18(17.48)
MSS	11(10.68)
MSI-high	16(15.53)
Subsite	4(3.887)
Cecum	25(24.27)
Ascending colon	29(28.16)
Transverse colon	

Demographic characteristics	Tissue n = 103(%)
Descending colon	
Sigmoid colon	
Rectum	

RESULTS

Patient Characteristics

In this study, a total of 103 colorectal cancer (CRC) cases were analysed (Table 2). The median age of patients was 69 years (range: 26–92). Tumour stages were distributed as follows: stage I (20%), stage II (26%), stage III (31%), and stage IV (23%).

HO-1 Expression and Clinicopathological Features

HO-1 expression was significantly associated with patient age, with higher expression observed in patients younger than 70 years ($p = 0.027$) (Table 3). HO-1 expression was significantly associated with MSI status ($p = 0.026$), with MSI-high tumours showing lower HO-1 expression than microsatellite-stable tumours. Furthermore, HO-1 expression demonstrated borderline statistical significance with LVI status ($p = 0.050$).

GPX4 Expression and Clinicopathological Features

GPX4 expression was significantly associated with age, with higher expression detected in patients younger than 70 years ($p = 0.008$) (Table 3). Also, GPX4 expression showed a trend toward association with T stage; however, statistical significance was not reached ($p = 0.058$).

SLC7A11 Expression and Clinicopathological Features

SLC7A11 expression was significantly associated with tumour anatomical subsite ($p = 0.037$) (Table 3). Higher expression was more frequently observed in proximal colon tumours (caecum, ascending, and transverse colon), whereas lower expression predominated in rectal tumours.

SLC7A11 Expression and Iron Levels

Tissue iron concentrations differed significantly according to *SLC7A11* expression status in CRC tissues ($p = 0.031$) (Table 4). The high-expression group exhibited a mean iron concentration of 77.29 ± 37.16 µg/g, whereas the combined low group showed a higher mean concentration of 92.57 ± 60.89 µg/g. This finding demonstrates an association between tissue iron concentration and *SLC7A11* expression in CRC.

Correlation Between Ferroptosis-Related Gene Expression

Evaluation of expression profiles demonstrated a strong association between HO-1 and GPX4 expression ($p < 0.001$), with approximately 62% of tumours exhibiting concordant expression. A trend toward association was observed between HO-1 and SLC7A11 expression ($p = 0.062$), although this did not reach conventional statistical significance. No significant relationship was identified between GPX4 and SLC7A11 expression.

Table 3
Correlations Between HO-1, GPX4 and SLC7A11 with Clinicopathological Characteristics of CRC

Variables	HO-1			GPX-4			SLC7A11		
	High expression n (%)	Low expression n (%)	P-value	High expression n (%)	Low expression n (%)	P-value	High expression n (%)	Low expression n (%)	P-value
Gender			0.56			0.39			0.36
Male	11(22.0)	39(78.0)		26(52.0)	24(48.0)		30(60.0)	20(40.0)	
Female	12(22.6)	41(77.4)		30(56.6)	23(43.4)		29(54.7)	24(45.3)	
Age			**0.027**			**0.008**			0.086
≤ 69 years	17(30.4)	39(69.6)		37(66.1)	19(33.9)		36(61.0)	23(39.0)	
> 69 years	6(12.8)	41(87.2)		19(40.4)	28(59.6)		20(45.5)	24(54.5)	
Size						0.52			0.56
≤ 40 mm	14(25.9)	40(74.1)	0.24	29(53.7)	25(46.3)		31(57.4)	23(42.6)	
> 40 mm	9(18.4)	40(81.6)		27(55.1)	22(44.9)		28(57.1)	21(42.9)	
Site			0.51			0.37			0.084
Colon	17(23.0)	57(77.0)		39(52.7)	35(47.3)		46(62.2)	28(37.8)	
Rectum	6(20.7)	23(79.3)		17(58.6)	12(41.4)		13(44.8)	16(55.2)	
Subsite			0.53			0.06			**0.037**
Cecum	3(16.7)	15(83.3)		10(55.6)	8(44.4)		12(66.7)	6(33.3)	
Ascending	4(36.4)	7(63.3)		8(72.7)	3(27.3)		6(54.5)	5(45.5)	
Transverse	3(18.8)	13(81.2)		6(37.0)	10(62.5)		10(62.5)	6(37.5)	
Descending	0(0.0)	4(100.0)		0(0.0)	4(100.0)		0(0.0)	4(100.0)	
Sigmoid	7(28.0)	18(72.0)		15(60.0)	10(40.0)		18(72.0)	7(28.0)	
Rectum	6(20.7)	23(79.3)		17(58.6)	12(41.4)		13(44.8)	16(55.2)	
Grade			0.15			0.09			0.47
Well-differentiated (1)	1(7.7)	12(92.3)		5(38.5)	8(61.5)		7(53.8)	6(46.2)	
Moderately differentiated (2)	21(26.2)	59(73.8)		48(60.0)	32(40.0)		48(60.0)	32(40.0)	
Poorly differentiated (3)	1(10.0)	9(90.0)		3(30.0)	7(70.0)		4(40.0)	6(60.0)	
T stage			0.31			0.058			0.47
I or II	12(25.5)	35(74.5)		30(63.8)	17(36.2)		16(55.2)	13(44.8)	
III or IV	11(19.6)	45(80.4)		26(46.4)	30(53.6)		43(58.1)	31(41.9)	
LVI			**0.05**			0.21			0.45
Negative	9(15.5)	49(84.5)		34(58.6)	24(41.4)		34(58.6)	24(41.4)	
Positive	14(31.1)	31(68.9)		22(48.9)	23(51.1)		25(55.6)	20(44.4)	

Variables		HO-1			GPX-4			SLC7A11	
	High expression n (%)	Low expression n (%)	P-value	High expression n (%)	Low expression n (%)	P-value	High expression n (%)	Low expression n (%)	P-value
Metastasis			0.64			0.16			0.42
M0	22(22.2)	77(77.8)		45(57.7)	33(42.3)		56(56.6)	43(43.4)	
M1	1(25.0)	3(75.0)		11(44.0)	14(56.0)		3(75.0)	1(25.0)	
Tumour Perforation			0.49			0.29			0.64
NO	21(21.9)	75(78.1)		51(57.7)	45(46.9)		55(57.3)	41(42.7)	
Yes	2(28.6)	5(71.4)		5(71.4)	2(28.6)		4(57.1)	3(42.9)	
MSI status			**0.026**			0.17			0.37
MSS	22(26.2)	62(73.8)		48(57.1)	36(42.9)		47(56.0)	37(44.0)	
MSI-high	1(5.3)	18(94.7)		8(42.1)	11(57.9)		12(63.2)	7(36.8)	

Table 4
Comparison of tissue iron concentrations according to SLC7A11 expression status in CRC tissues.

SLC7A11 expression	n (%)	Iron concentration (μg/g), mean ± SD
High expression	34(73.9)	77.29 ± 37.16
Low expression	12(26.1)	92.57 ± 60.89
P-value	**0.031**	

Table 5
Correlation Among HO-1, GPX4 and SLC7A11 in CRC

Variables		GPX-4			SLC7A11	
	High expression n (%)	Low expression n (%)	P-value	High expression n (%)	Low expression n (%)	P-value
HO-1			**< 0.001**			**0.062**
High expression n (%)	20(87.0)	3(13.0)		17(73.9)	6(26.1)	
Low expression n (%)	36(45.0)	44(55.0)		42(52.5)	38(47.5)	

DISCUSSION

The present study provides comprehensive evidence linking ferroptosis-related genes (HO-1, GPX4, and SLC7A11) with clinicopathological features and iron metabolism in colorectal cancer (CRC), highlighting their potential role in tumour biology and therapeutic resistance.

One of the key findings of this study is the significant association between HO-1 expression and patient age, with higher expression observed in younger patients. Wang et al. also found a correlation with high HO-1 expression in thyroid cancer (Wang, Liu et al. 2015). Additionally, HO-1 expression showed a borderline association with lymphovascular invasion (LVI), with reduced HO-1 expression more frequently observed in LVI-positive tumours. Although not statistically significant, the observed trend raises the possibility that reduced HO-1 expression may be associated with a more invasive tumour parameter.

HO-1 has dual functions as a cytoprotective molecule and a regulator of tumour progression (Wang, Cheng et al. 2023). HO-1 expression may increase oxidative stress and genomic instability, promoting tumour invasion and dissemination through lymphatic and vascular channels (Chiabrando, Vinchi et al. 2014, Loboda, Damulewicz et al. 2016). In agreement with our findings, HO-1-positive CRC have been associated with lower lymphatic invasion and improved overall survival (Becker, Fukui et al. 2007). Moreover, loss of HO-1 may enhance chronic inflammation within the tumour microenvironment, further contributing to tumour progression and invasion(Nitti, Piras et al. 2017).

However, the role of HO-1 in cancer remains complex and appears to be highly context-dependent. In contrast to CRC, studies in non-small-cell lung cancer have reported that increased HO-1 expression is associated with lymph node metastasis, advanced tumour stage, and poor prognosis, suggesting that HO-1 may promote tumour progression in certain malignancies(Tsai, Wang et al. 2012). These contrasting findings reflect the multifaceted roles of HO-1 and its potential as a therapeutic target in cancer.

A significant association was identified between HO-1 expression and MSI status, with MSI-high tumours demonstrating lower HO-1 expression. The underlying mechanisms contributing to this relationship remain unclear; however, the unique molecular landscape and immune microenvironment characteristic of MSI-high colorectal cancers may modulate oxidative stress responses and redox-regulatory pathways [23]. Further studies are needed to define the biological and clinical relevance of HO-1 expression in the context of mismatch repair-deficient colorectal cancer.

In the present study, GPX4 expression was significantly higher in younger patients, suggesting a potential age-related influence on ferroptosis regulation in colorectal cancer. GPX4, a selenium-dependent phospholipid hydroperoxidase, functions as the primary suppressor of ferroptosis by reducing membrane lipid hydroperoxides in a glutathione-dependent manner. Through limiting lipid peroxidation and maintaining membrane integrity, GPX4 protects cells from iron-dependent oxidative cell death, potentially promoting tumour cell survival in younger CRC cohorts (Dixon, Lemberg et al. 2012, Stockwell, Angeli et al. 2017).

Growing evidence indicates that aberrant GPX4 expression contributes to tumour initiation, progression, and therapeutic resistance across multiple malignancies, with elevated GPX4 frequently associated with poor clinical outcomes (Li, Qian et al. 2025, Shimura, Yin et al. 2025, Ye, Zhang et al. 2025). In CRC, Oe et al. demonstrated that increased GPX4 expression is significantly associated with lymph node metastasis and advanced TNM stage, supporting its role in tumour progression (Ou, Wu et al. 2024). Similarly, in epithelial ovarian cancer, high GPX4 expression has been linked to platinum resistance, suggesting that GPX4-mediated suppression of ferroptosis contributes to chemotherapy resistance (Wu, Shen et al. 2022). In non-small cell lung cancer, GPX4 has also been implicated in maintaining cancer stem cell populations and promoting radioresistance, further highlighting its role in tumour aggressiveness and treatment failure(Kim, Min et al. 2023). The observed association between higher GPX4 expression and younger patients in our cohort may reflect age-related differences in redox homeostasis or tumour biology. Enhanced GPX4 expression could provide greater protection against ferroptosis (Stockwell 2022), thereby facilitating tumour cell survival and disease progression in younger individuals. Although the underlying mechanisms remain unclear, these findings suggest that age may influence ferroptosis regulation in colorectal cancer and warrant further investigation in larger, mechanistic studies.

Notably, SLC7A11 expression was significantly associated with tumour subsite, with higher expression observed in proximal colon tumours. Given its role in maintaining redox homeostasis and suppressing ferroptosis, elevated SLC7A11 expression may promote tumour survival and therapeutic resistance. (Cheng, Wang et al. 2022). This association may reflect the distinct molecular characteristics of proximal colorectal cancers, including differences in MSI status and immune activation(Abida, Cheng et al. 2019). Further studies are needed to determine whether ferroptosis susceptibility varies according to tumour location.

A key finding of this study was the significant association between SLC7A11 expression and tissue iron concentrations in CRC. Tumours with low SLC7A11 expression exhibited higher mean iron concentrations than those with high expression ($p = 0.031$), suggesting a potential relationship between iron homeostasis and ferroptosis-related antioxidant defences. As a component of system Xc^-, SLC7A11 promotes cystine uptake and glutathione synthesis, supporting GPX4-mediated detoxification of lipid hydroperoxides and protection against ferroptosis (Sun, Zhu et al. 2023). The lower iron concentration observed in the high-SLC7A11 group may reflect differences in iron regulation or cellular redox status. However, as total tissue iron measurements do not distinguish redox-active iron from stored iron, the biological basis of this association remains unclear. Further studies evaluating labile iron, GSH status, GPX4 activity, and lipid peroxidation are needed to clarify the relationship between iron homeostasis and ferroptosis-related antioxidant defences.

Correlation analysis further demonstrated a strong positive association between HO-1 and GPX4 expression, indicating coordinated regulation of iron metabolism and antioxidant defence pathways in colorectal cancer. HO-1 catalyses the degradation of haem into biliverdin, carbon monoxide, and ferrous iron. While biliverdin and its metabolite bilirubin possess potent antioxidant properties, the release of ferrous iron may simultaneously increase the intracellular labile iron pool and promote lipid peroxidation. Consequently, tumour cells experiencing enhanced HO-1 activity may require increased GPX4 expression to detoxify iron-induced lipid hydroperoxides and prevent ferroptosis(Li, Qian et al. 2025, Pham, Refesse et al. 2025). This coordinated expression may therefore represent a compensatory mechanism that allows tumour cells to tolerate persistent oxidative stress while maintaining viability. Similar interactions between HO-1-mediated iron metabolism and GPX4-dependent antioxidant defence have been described in several experimental models of ferroptosis, supporting the biological plausibility of our findings (Deng, Yue et al. 2025, Li, Qian et al. 2025).

Despite the observed associations with age, MSI status, tumour subsite, and iron levels, no significant relationships were identified between ferroptosis-related markers and overall tumour stage, grade, or metastatic status. This suggests that alterations in ferroptosis-associated pathways may occur early during colorectal tumorigenesis and are not necessarily linked to tumour burden or disease extent.

This study has also identified a marginal association between HO-1 and SLC7A11 expression. A potential explanation is the activation of the nuclear factor erythroid 2-related factor 2 (NRF2) pathway, the principal transcriptional regulator of the cellular antioxidant response. Under oxidative stress, NRF2 translocates to the nucleus and induces the transcription of multiple cytoprotective genes, including HMOX1 (encoding HO-1) and SLC7A11(Dong, Qiang et al. 2020). Coordinated upregulation of these genes enhances the cellular capacity to detoxify ROS, maintain glutathione homeostasis, and suppress ferroptosis. Therefore, the observed correlation between HO-1 and SLC7A11 may reflect activation of a common NRF2-dependent adaptive programme rather than a direct regulatory interaction between the two genes(Gelerstein-Claro, Espinoza-Jaña et al. 2025). Persistent activation of this pathway has been implicated in tumour progression, therapeutic resistance, and poor clinical outcomes across multiple malignancies(Dong, Qiang et al. 2020, Yao, Bao et al. 2024).

In conclusion, this study demonstrates significant associations between ferroptosis-related genes and selected clinicopathological features of colorectal cancer, including age, MSI status, tumour subsite, and tissue iron levels. The observed relationships among HO-1, GPX4, and SLC7A11 support the concept that ferroptosis-associated pathways contribute to the maintenance of redox homeostasis in colorectal tumours. These findings provide additional evidence linking iron metabolism and ferroptosis in colorectal cancer and identify potential molecular targets for future ferroptosis-based therapeutic strategies. Further mechanistic and longitudinal studies are required to establish the functional and clinical significance of these biomarkers.

Limitation

This study has several limitations. Notably, owing to insufficient tumour tissue, tissue iron concentration could be quantified by ICP-OES in only 46 of the 103 colorectal cancer cases. Although this subset remained representative of the study cohort, the reduced sample size may have limited the ability to detect weaker associations involving tumour iron levels. Further studies with larger sample sizes and complete iron profiling are needed to confirm these findings.

Declarations

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The authors declare that this study did not receive any funding.

Author Contribution

N.M: Writing – original draft, Methodology, Investigation, Data curation, Conceptualisation. G.V.: Investigation, Data curation. Z. CH.: Methodology, Investigation. M.S.I.: Methodology, Investigation. J.Y.W.: Investigation, Data curation .M .N.: Methodology, Investigation: C.T.L.: Resources, Investigation. A.D.: Methodology. V. G.: Writing – review & editing, Supervision, Project

administration, Methodology, Conceptualisation. A.K. L.: Writing – review & editing, Supervision, Resources, data analysis, Project administration. All authors read and approved the final manuscript.

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