Intravenous Iron Therapy Replenishes Iron Stores without Enhancing Tumour Responses in Anaemic Patients with Colorectal Cancer: Comparison with Oral Iron Supplementation.

Hafid O Al-Hassi
University of Wolverhampton https://orcid.org/0000-0002-5119-7793
Oliver Ng
University of Nottingham School of Medicine
Rayko Evstatiev
Medizinische Universitat Wien
Manel Mangalika
Royal Wolverhampton Hospitals NHS Trust
Natalie Worton
Royal Wolverhampton Hospitals NHS Trust
Manuela Jambrich
Royal Wolverhampton Hospitals NHS Trust
Vineeta Khare
Medizinische Universitat Wien
Oliver Phipps
University of Wolverhampton
Barrie Keeler
University of Nottingham School of Medicine
Christoph Gasche
Medizinische Universitat Wien
Austin G Acheson
University of Nottingham School of Medicine
Matthew J. Brookes (matthew.brookes@nhs.net)
Royal Wolverhampton Hospitals NHS Trust

Research

Keywords: Iron deficiency anaemia, Oral iron treatment, intravenous iron treatment, colorectal cancer

Posted Date: August 1st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-48784/v1

Abstract

Background: Oral iron promotes intestinal tumourigenesis in animal models. In humans, expression of iron transport proteins are altered in colorectal cancer. This study examines whether the route of iron therapy alters iron transport and tumour growth.

Methods: Colorectal adenocarcinoma patients with pre-operative iron deficiency anaemia received oral ferrous sulphate (n=15), or intravenous ferric carboxymaltose (n=15). Paired (normal and tumour tissues) samples were compared for expression of iron loading, iron transporters, proliferation, apoptosis, Wnt signalling pathway and microsatellite instability using immunohistochemistry and RT-PCR.

Results: Iron loading was increased in tumour and distributed to the stroma in intravenous treatment and to the epithelium in oral treatment. The protein and mRNA expression of iron transporters were increased in tumours compared to normal tissues but there were no significant differences between the treatment groups. However, intravenous iron treatment reduced ferritin mRNA levels in tumours ($p<0.001$) and successfully replenished body iron stores without increasing tumour growth, DNA damage markers, proliferation or apoptosis compared with oral iron treatment.

Conclusion: Iron distribution to non-epithelial cells in intravenous iron treatment suggests that iron is less bioavailable to tumour cells. Therefore, intravenous iron supplementation could be a safer option in the treatment of colorectal cancer patients with iron deficiency anaemia due to the differential compartmentalisation of iron within the intestinal mucosa and its efficiency in replenishing body iron levels without increasing the risk of tumour growth.

Trial registration: The study was registered with the Medicines and Healthcare Regulatory Agency, clinical trials.gov (NCT01927328) and EudraCT (2013-000209-22).

Introduction

Iron is a vital element for many biological functions including oxygen delivery, metabolism, growth and DNA synthesis [1,2]. Excess iron can create reactive oxygen species which can induce mutation of the mismatch repair genes and subsequently leads to microsatellite instability (MSI) causing DNA damage and carcinogenesis [3].

Cellular absorption of dietary non-haem iron occurs in the duodenum and upper jejunum via the duodenal cytochrome b-like ferrireductase (DcytB) [4] and imported into the cell by the divalent metal transport 1 (DMT1). However, these cellular iron transporters are now also known to be present within the colonic epithelium [5] and are modified in CRC [6].

After absorption iron is either stored as ferritin or exported from cells via the basolateral ferroportin (FPN) [7,8] facilitated by the membrane protein hephaestin (HEPH) or plasma protein ceruloplasmin. Iron can then be transported in the extracellular fluid and plasma, bound to transferrin [9]. Cells then obtain iron

via binding of the iron-transferrin complex to transferrin-receptor 1 (TfR1) [10,11] and released from the endosome via DMT1 to again form a labile iron pool, which can be taken up for the cellular processes [12].

Cellular iron transporter levels are controlled at a post-transcriptional level by iron-responsive binding proteins (IRP) 1 and 2 [13]. When activated by iron-deficiency, IRPs bind to iron-responsive elements (IREs) in the untranslated regions of messenger RNA including TfR1 and ferritin and promote the translation of TfR1 and repression of ferritin which increases the labile intracellular iron pool with decreases in iron export, utilisation and storage [14,15].

Colorectal cancer (CRC) is a major cause of cancer-related mortality worldwide [16] and associated with iron deficiency anaemia (IDA). Oral iron treatment of anaemia may prove inappropriate because colonic iron is implicated in gut mucosal inflammation, CRC growth and stimulation of oxidative stress [17-20]. Studies in animal models showed that high dietary iron induced intestinal inflammatory responses, impaired intestinal immune and barrier function [21] and CRC growth in mice in the presence of the colonotropic carcinogen, azoxymethane [22]. However, most studies on the association between dietary iron intake and development of CRC were conducted in animal models or on cell lines using supra-physiological doses of iron. One cohort study found no association between dietary iron and the risk of CRC in women [23]. On the other hand, systemic iron replacement does not increase carcinogenesis despite adequately replenishing iron stores with high profile of safety and tolerance [24-25]. However, in humans the consequences of elevated luminal or systemic iron on CRC still not well understood [26]. We hypothesize that the route of administration of iron supplementation affects iron distribution within the colonic epithelium and thus alters tumour cell biology. This work will examine the route of iron therapy on mucosal iron distribution and evaluate its effects on tumour cells.

Methods

Samples

The IVICA (IVICA; IntraVenous Iron in CRC associated Anaemia) is a multi-centre control trial that recruited anaemic adult patients with non-metastatic colorectal adenocarcinoma. Patients were randomised to receive either oral ferrous sulphate (OI) or intravenous ferric carboxymaltose (IV) for at least two weeks before surgery [27]. Intraoperative tissue (colorectal adenocarcinoma and paired normal tissue) were collected.

Immunohistochemistry

Paired paraffin embedded normal and tumour tissues (n=30/group) were dewaxed and rehydrated. Endogenous enzymes were blocked with 15% H_2O_2 in methanol. Antigens were retrieved by heat induction and non-specific binding was blocked using normal goat serum. Primary antibody (Additional file 1) was added for overnight at 4°C. Slides were washed with TRIS buffer and biotinylated secondary

antibody was added for 30 minutes. The avidin-biotin complex (Vector Laboratories, UK) was used and staining was visualized using 3,3'-diaminobenzidine (Fluka, UK). Sections were counterstained with hematoxylin and mounted under coverslip. Secondary antibody alone was used as control. Images were taken on an Olympus BX51 microscope. Quantitative analysis was performed on target proteins (Additional file 2) blind to the treatment. Ratio of positive cells or an analysis of immuno-reactivity with intensity scored from 0-2 was also determined. Tumours were analysed for MLH1 and MSH2 loss to determine microsatellite instability (MSI) and microsatellite stability (MSS) phenotypes.

Perls Prussian blue staining

Tissue sections (n=30/group) were rehydrated as per immunohistochemistry. Solution of 0.7g ferrocyanide in 70 ml 0.5% HCl (HT20, Sigma, UK) was applied at room temperature for 60 minutes. Counterstain with nuclear fast red was performed for 1 minute. Five high magnification fields were assessed per sample and an average score of staining was calculated. Assessors were blinded to the treatment received.

RT-PCR

RNA was extracted from snap frozen tissue (n=30/group) using the Thermo-Fisher Scientific mirVana™ miRNA isolation kit. Organic extraction was achieved with acid phenol chloroform and RNA purity and concentration were then determined using a NanoDrop™ 2000/2000c Spectrophotometers (ThermoFisher). Synthesis of cDNA was performed using the Qiagen® QuantiTect® Reverse Transcription kit. Genomic DNA was eliminated, and template RNA was mixed with reverse-transcription master-mix and incubated at 42°C for 15 minutes, denatured at 95°C and stored at -20°C. RT-PCR was performed for MYC, IRP2, FTH1, TFRC, SLC11A2 using GAPDH control as an internal standard (Thermo Fisher Scientific). Probes and primers are listed in (Additional file 3). TaqMan™ Gene expression master mix (Thermo Fisher Scientific) containing AmpliTaq Gold® DNA Polymerase (Ultra-Pure), Uracil-DNA glycosylase, dNTPs with dUTP, ROXTM Passive Reference and optimised buffer components, was added to 100 ng of cDNA and dH_2O to form a 25 microL reaction mixture. Reactions without cDNA were included as negative controls. Reactions were performed in triplicate and RT-PCR was conducted using a 7500 Fast Real Time PCR System. Gene expression was normalised to GAPDH, represented as ΔCt and compared between tumour and paired normal tissues to give a ΔΔCt value. Changes in gene expression were represented a negative log of ΔΔCt and 1 regarded as normal.

Statistics

Paired t test was used to test for significance between normal and tumour tissue, and between pre-treatment and post-treatment. Independent t test was used to compare between treatment groups. Linear regression analysis was performed compare mRNA expression and statistical significance tested with

Pearson correlation coefficient. Chi square was used to analyse iron expression in tissue sections. A p-value of <0.05 was considered significant.

Results

Clinical outcome

In the selected subset of patients from the IVICA trial, all patients reported compliance with OI therapy. Patients were similar at recruitment for age, sex, Dukes stage, haemoglobin, ferritin and transferrin saturations. Patients had a significantly higher increase in ferritin in the IV group (median ferritin 588 ng/mL IV versus 22 ng/mL oral, p=0.001). Haemoglobin and transferrin saturations were also higher in the IV group by day of surgery (Table 1).

Table 1
Clinical outcome

	Oral iron (n=15)	IV iron (n=15)	p
Age (years)[1]	74 (46-82)	74 (53-85)	0.662
Sex ratio (M:F)	10:5	8:7	0.758
Haemoglobin (g/dL)[2]			
Recruitment	10.3 (1.0)	10.0 (1.7)	0.616
Day of surgery	11.4 (1.1)	12.3 (2.1)	0.131
Ferritin (ng/mL)[3]			
Recruitment	21 (14-45)	39 (12-204)	0.384
Day of surgery	22 (16-51)	588 (318-1415)	0.001*
Transferrin saturations (%)[3]			
Recruitment	7 (4-16)	5.5 (2-14)	0.301
Day of surgery	7.5 (4-13)	20 (17-24)	0.290
Dukes n (%)			
A	0	2 (13.3)	0.845
B	9 (60)	10 (66.7)	
C	6 (40)	3 (20)	
Site n (%)			
Caecum	6 (40)	9 (60)	
Ascending colon	0	0	0.938
Hepatic flexure	1 (6.7)	1 (6.7)	
Transverse colon	3 (20)	0	
Splenic flexure	2 (13.3)	1 (6.7)	
Descending colon	0	1 (6.7)	
Sigmoid	2 (13.3)	0	
Rectum	1 (6.7)	3 (20)	
Iron therapy	Ferrous sulphate 200mg BD PO	Ferric carboxymaltose 1000mg Single dose IV	-
	25 (16-36)	26 (15-34)	0.798

Days before surgery[1]			
Transfusions	0	0	-
1 Median (range) 2 Mean (Standard deviation) 3 Median (Interquartile range) * p<0.05.			

Patients in the OI group remained iron deficient, ferritin 22 ng/ml. No patients had a pre-operative transfusion (Fig.1).

Microsatellite instability

MSI was present in 23% of tumours, with five cancers demonstrating loss of MLH1 and two cancers demonstrating loss of both MSH2 and MLH1. Of these seven tumours, four MSI tumours were in the OI group and the remaining three in the IV group. MSS and MSI tumours were compared for all RT-PCR and immunohistochemistry outcomes and across treatment groups (Additional file 4 and 5). Sub-analyses are discussed below.

Cellular proliferation and Wnt signalling

No significant differences between tumours in oral and IV groups were seen. Exclusion of MSI phenotype did not alter results between treatment groups, (Additional file 4 and 5).

The proliferation marker Ki67 immune staining was mainly nuclear with low immuno-reactivity in normal tissue but significantly higher in tumours (p=0.002) (Fig. 2a and b). No difference was seen between treatment groups.

Membranous and cytoplasmic β-Catenin, the main intracellular signal transducer in the Wnt signalling pathway, had immunoreactivity in both normal and tumour tissues. However, nuclear staining was only seen in some tumour tissues and no normal tissues (Fig. 2c).

There were no significant differences in β-Catenin membranous expression between normal and tumour tissues. In contrast, both cytoplasmic and nuclear immunoreactivity for β-Catenin showed significant differences between normal and tumour tissue (p<0.001) (Fig. 2d). No differences were seen between treatment groups. The p21-activated kinase 1 (PAK1), a downstream effector of GTPases overexpressed in many tumours, showed cytoplasmic immunoreactivity in all tissues, both normal and tumour (Fig. 2e). There were no significant differences between normal and tumour or between treatment groups.

Wnt signalling target gene c-MYC mRNA fold-changes were significantly higher in tumour cells compared with normals (p<0.0001) and increased in both treatment groups (Fig. 2f). IRP2 mRNA levels positively correlated with MYC mRNA levels (R2=39%, ANOVA p=0.010) and SLC11A2 (R2=67%, p<0.001) (Fig. 2g).

DNA damage and apoptosis

The tumour suppressor genes p53 revealed no positive immunoreactivity in the nuclei in 89% of normal tissue. In comparison, only 20% of tumours had no positive nuclei (Fig. 3a) and there was a statistically significant difference in immunoreactivity between normal and tumour tissue ($p<0.002$) (Fig. 3b). There were no significant differences in p53 expression between the treatment groups. MSI tumours were associated with higher p53 expression compared to the MSS group ($p=0.01$) (Additional file 5). DNA double strand breaks, detected through γH2AX protein staining showed no or low nuclear immuno-reactivity in all normal tissue. Tumour, as expected, had higher immuno-reactivity (Fig. 3c). There was a statistically significant difference between normal and tumour tissues ($p<0.0001$) (Fig. 3d). No difference was seen between treatment groups in normal or tumour tissue. There was no positive staining for the apoptosis marker cleaved caspase 3 (CC3) protein in normal tissue. However, all tumours except one had some CC3 immunoreactivity (Fig. 3e) which was statistically significant compared to that of normal tissues (Fig. 3f). There were no differences between treatment groups.

Tissue iron loading and storage

Iron loading was significantly increased in tumour tissues compared to normal tissues from the OI group ($p=0.005$) (Fig. 4a and b). similar staining was observed between the normal and tumour tissues from the IV group (data not shown). In tumour tissues, Perls Prussian blue expression was significantly higher in the epithelial cells from the oral group compared with the IV group ($p=0.01$) whereas in the IV group it was significantly distributed to the stroma ($p=0.0001$) (Fig. 4c). FTH1 mRNA that encodes the heavy subunit of ferritin, was reduced in tumours compared to normal tissues with a greater reduction in the IV group, $p<0.001$ (Fig. 4d), (Additional file 6).

Cellular iron transport and iron regulation

The iron transport gene SLC11A2 expression in OI versus IV and the immunoreactivity of its encoded protein DMT1 were higher in tumours compared to normal tissues but this increase did not reach statistical significance ($p<0.07$) (Fig. 5a - c respectively). In OI compared to IV, RT-PCR showed IRP2 (IREB2) was reduced (Fig. 5d) and MSS versus MSI groups were significantly different with an increased IRP2 ($p=0.001$) and SLC11A2 ($p<0.009$) in the MSI group, (Additional file 4). IRP2 mRNA levels positively correlated with SLC11A2 ($R^2=67\%$, $p<0.001$, Fig. 5e).

TFRC gene expression in OI treatment was not significantly different to that in IV treatment group (Fig. 6a). However, the immunoreactivity of its protein TfR1 was significantly higher in tumour tissues compared with normal tissues ($p<0.0001$) (Fig. 6b and c respectively). Furthermore, immune-reactivity of ferroportin was significantly higher in tumour compared to normal tissue ($p<0.001$) (Fig. 6d and e respectively). Staining for DMT1, TfR1 and ferroprotin was altered in tumours and localised to the cytoplasm whereas in normal tissues their expression was membranous (Fig. 5b, 6b and 6d respectively).

No significant differences in the iron transporters were seen between treatment groups and sub-analysis of microsatellite instability did not alter these results.

Discussion

This study examined for the first time in humans, two groups of anaemic patients with CRC, randomised to oral or IV iron therapy. It compared molecular changes between normal and tumour tissue and between treatment groups. IV iron therapy successfully replenished body iron, with increases in ferritin and transferrin saturation. The OI group remained iron deficient.

Despite increase in tumour and body iron, this did not lead to increased proliferation or decreased apoptosis in tumours in the IV group when compared to the OI group and was not associated with changes in proliferation in the paired normal tissues. These findings suggest that IV iron does not increase tumour progression and development and could be a safe alternative to OI. Studies in mouse models of colitis-associated CRC by contrast, showed OI increases the number and size of tumours when compared to an iron-deficient diet and IV [24]. However, this model of inflammatory colorectal carcinogenesis is unlike most sporadic colorectal cancers in humans. Further, our human participants were still consuming a normal Western diet commonly replete in dietary iron, rather than the experimental iron-deficient diet of the mouse in Seril et al study [24]. Higher supra-physiological doses of iron were also administered to mice in comparison, over a relatively longer time period when compared to the time period over which carcinogenesis occurs in humans.

Results are in agreement with a previous study by Brookes et al (2006) [6]. However, qualitative differences between the localisation of iron within the stroma and adjacent connective tissues were noted, occurring more frequently with IV. The implications of this are uncertain but may indicate that less iron is available to tumour cells from patients treated with IV iron. Further, differential compartmentalisation and the tumour microenvironment could all potentially influence intracellular tumour iron loading, macrophage iron and immune function. However, differential compartmentalisation of iron within tissue should also be interpreted carefully. Haemosiderin stains intensely with Prussian blue and ferritin only at high concentrations [28]. Haemosiderin may be largely inert and biologically inactive, reflecting instead a secondary mechanism for iron storage when ferritin storage is exceeded [29]. The biologically active labile iron pool however is not seen or quantified with Prussian blue and has instead been inferred.

Iron importers TfR1 and DMT1 at both mRNA and protein level were increased in tumours with no differences seen between treatment groups. Ferritin heavy chain mRNA was also reduced. The net effect of decreased iron storage and increased iron import would be an increase in the labile iron pool. This appears to be occurring due to a change of normal iron sensing mechanisms. IRP2 was decreased in tumours, a normal response to high intracellular iron, but this did not lead to a reduction in TfR1 or an increase in FTH1 as expected. In fact, TfR1 expression increased and FTH1 mRNA expression decreased

with no correlation with IRP2 mRNA expression. This is contrary to findings by Horniblow (2017) in which IRP2 and TfR1 expression both increased in CRC and correlated with each other [30].

This effect had previously been demonstrated in relationship to APC wild type cancer cell lines, whereby the regulation of iron stores appeared to be IRE/IRP dependent with normal iron decreasing IRP2 with subsequent decreases in TFR1 and DMT1 [31]. In cancers with a mutation in APC, the regulation of colon cancer cells iron stores became IRE/IRP independent and despite high iron, TFR1 and DMT1 expression increased. This could be reversed when APC was transfected into these cells [31]. This model would hypothesise that IRE/IRP sensing might be bypassed by beta-catenin TCF signalling and overwhelmed by huge increases in iron. In this study, there was no difference in iron regulation between MSI and MSS (and likely APC pathway) tumours.

Changes in iron metabolism were also not related to c-MYC expression, which correlated with IRP2 but showed no relationship with iron transport (SLC11A2 and TFRC) or storage (FTH1). Previous in vitro experiments examining transcriptional targets of c-MYC have yielded mixed results. One study has shown increased c-MYC causes an overexpression of IRP2 and a reduction of FTH1 but had no effect on TFRC [32]. Others have shown that c-MYC can independently induce TfR1[33] and that transfection of c-MYC to colon cancer cell lines increases ferritin heavy chain transcription [34].

Iron export via ferroportin was also altered in tumours with increased expression and mis-localisation from the membrane to the cytoplasm, the latter potentially reducing iron export from cells again increasing labile iron.

Limitations to this study include the likelihood of marked heterogeneity in the tumours and treatment despite matching for tumour stage, histology and sub-analysis by MSI status. Also, heme iron pathways play a smaller but significant role in iron absorption and are neither controlled for nor examined in this study. The small window of intervention, just over two weeks, may also be insufficient to alter the biology of the tumour. Nevertheless, this short period of exposure reflects the real-life use of iron replacement therapy in patients with CRC. However, in view of these preliminary findings, further investigations are warranted to confirm the efficacy of IV iron therapy in CRC patients without promoting tumour progression.

In conclusion, IV is more effective in correcting iron deficiency and clinical anaemia [24, 35]. This study has investigated the molecular consequences of IV treatment and has shown no changes in tumour growth despite iron avid tumours and therefore supports the safety of iron replenishment of body iron stores with IV iron.

Abbreviations

c-MYC: c-myelocytomatosis; CC3: Cleaved caspase-3; CRC: Colorectal Cancer; DcytB: Cytochrome b-like ferrireductase; DMT1: Divalent metal transport 1; FPN: Ferroportin; FTH1: Ferritin heavy chain; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; HEPH: Hephaestin; IDA: Iron deficiency anaemia; IRE: Iron-

responsive elements; IRP: Iron-responsive binding proteins; IV: Intravenous iron; Microsatellite instability (MSI); Microsatellite stability (MSS); OI: Oral iron; SLC11A2: DMT-1 encoding protein; TfR1: Transferrin-receptor 1; TFRC: Transferrin-receptor 1 encoding gene.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki (2000) of the World Medical Association with ethical approval (13/EM/0069) NRES Committee East Midlands - Nottingham 2, UK.

Consent for publication

All authors consented to the publication of this article.

Availability of data and materials

Data generated or analysed during this study are included in this published article [and its supplementary information files] and are also available from the corresponding author on reasonable request.

Competing interests

HOA, ON, MM, NW, MJ, OP, VK and BK declare that they have no conflict of interest. MJB research department has received grant support from Tillotts Pharma and Vifor Pharma (Switzerland). MJB has received honoraria and travel support for consulting or lecturing from Vifor Pharma, Tillotts Pharma and Abbvie. AA research department has received grant support from Pharmacosmos, Denmark and Vifor Pharma, Switzerland. Honoraria or travel support received for lecturing from the following companies: Olympus, Essex, UK., Vifor Pharma Ltd, Switzerland and Pharmacosmos, Denmark. CG: Advisory Board/Consultant/Speaker's Bureau: Fresenius Medical Care, Pharmacosmos A/S, Renapharma Sweden, Vifor Int.; Grant/Research Support: AOP Orphan Pharmaceuticals, Biogena Naturstoffe GmbH. RE: Advisory Board for AOP Orphan Pharmaceuticals, Speaker's honoraries from Vifor Pharma Int and AOP Orphan Pharmaceuticals.

Funding

This study was funded by the Rotha Abraham Bequest, the Royal Wolverhampton NHS Trust, Wolverhampton, UK. The Medical Scientific Fund of the Mayor of the City of Vienna, Vienna, Austria.

Authors’ contributions

Hafid O Al-Hassi, Oliver Ng, Rayko Evstatiev, Barrie Keeler, Christoph Gasche, Austin G Acheson, and Matthew J Brookes designed, performed, analyzed experiments and drafted the manuscript. Manel Mangalika, Natalie Worton, Manuela Jambrich, Vineeta Khare and Oliver Phipps provided technical and material support and were involved in the acquisition and interpretation of data. All authors commented on previous versions of the manuscript, read and approved the final manuscript.

References

1. Abbaspour NR, Hurrell R, Kelishadi R. Review on iron and its importance for human health. J Res Med Sci. 2014;19(2):164-174.
2. Evstatiev R and Gasche C. Iron sensing and signalling. Gut. 2012;6:933-952.
3. Bridge G, Rashid S and Martin SA. DNA mismatch repair and oxidative DNA damage: implications for cancer biology and treatment. Cancers (Basel). 2014;5:1597-1614.
4. Frazer DM, Wilkins SJ, Vulpe CD, Anderson GJ. The role of duodenal cytochrome b in intestinal iron absorption remains unclear. Blood. 2005;106:4413-4414.
5. McKie A.T, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science. 2001;29:1755-1759.
6. Brookes MJ, Hughes S, Turner FE, Reynolds G, Sharma N, Ismail T, et al. Modulation of iron transport proteins in human colorectal carcinogenesis. Gut. 2006;55:1449-1460.
7. McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, et al. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol Cell. 2000;5:299-309.
8. Brittenham GM. Pathophysiology of Iron Homeostasis. In: E.J.B.J. Ronald Hoffman, Leslie E Silberstein, Helen E Heslop, Jeffrey I Weitz, John Anastasi, Editors. Hematology: Basic Principles and Practice. Philadelphia: Elselvier Saunders; 2013. p. 427-437.
9. Aisen P. Transferrin receptor 1. Int J Biochem Cell Biol. 2004;36:2137-2143.
10. Jeffrey GP, Basclain KA, Allen TL. Molecular regulation of transferrin receptor and ferritin expression in the rat gastrointestinal tract. Gastroenterology. 1996;110:790-800.
11. Lee AW, Oates PS, Trinder D. Effects of cell proliferation on the uptake of transferrin-bound iron by human hepatoma cells. Hepatology. 2003;38:967-977.
12. Breuer W, Shvartsman M, Cabantchik ZI. Intracellular labile iron. Int J Biochem Cell Biol. 2008;40:350-354.
13. Miyazawa M, Bogdan AR, Hashimoto K, Tsuji Y. Regulation of transferrin receptor-1 mRNA by the interplay between IRE-binding proteins and miR-7/miR-141 in the 3'-IRE stem-loops. RNA. 2018;24:468-479.

14. Zhang DL, Ghosh MC, Rouault TA. The physiological functions of iron regulatory proteins in iron homeostasis - an update. Front Pharmacol. 2014;5:124.
15. Wilkinson N and Pantopoulos K. The IRP/IRE system in vivo: insights from mouse models. Front Pharmacol. 2014;28;5:176.
16. Padmanabhan H, Brookes MJ, Iqbal T. Iron and colorectal cancer: evidence from in vitro and animal studies. Nutr Rev. 2015;73:308-317.
17. Nelson RL. Iron and colorectal cancer risk: human studies. Nutr Rev. 2001;59:140-148.
18. Chua AC, Klopcic B, Lawrance IC, Olynyk JK, Trinder D. et al. Iron: an emerging factor in colorectal carcinogenesis. World J. Gastroenterol. 2010;16: 663-672.
19. Chua AC, Klopcic BR, Ho DS, Fu SK, Forrest CH, Croft KD, et al. Dietary iron enhances colonic inflammation and IL-6/IL-11-Stat3 signaling promoting colonic tumor development in mice. PLoS One. 2013; doi:10.1371/journal.pone.0078850.
20. Thomas G. DeLoughery. Safety of Oral and Intravenous Iron. Acta Haematol. 2019;142(1):8-12.
21. Luo Q, Lao C, Huang C, Xia Y, Ma W, Liu W, et al. Iron Overload Resulting from the Chronic Oral Administration of Ferric Citrate Impairs Intestinal Immune and Barrier in Mice. Biol Trace Elem Res. 2020; doi:10.1007/s12011-020-02218-4.
22. Ilsley JN, Belinsky GS, Guda K, Zhang Q, Huang X, Blumberg JB, et al. Dietary iron promotes azoxymethane-induced colon tumors in mice. Nutr Cancer. 2004;49:162-9.
23. Kabat GC, Miller AB, Jain M, Rohan TE. A cohort study of dietary iron and heme iron intake and risk of colorectal cancer in women. Br J Cancer. 2007;97:118-122.
24. Seril DN, Liao J, Yang CS, Yang GY. Systemic iron supplementation replenishes iron stores without enhancing colon carcinogenesis in murine models of ulcerative colitis: comparison with iron-enriched diet. Dig Dis Sci. 2005;50:696-707.
25. Auerbach M, Gafter-Gvili A, Macdougall IC. Intravenous iron: a framework for changing the management of iron deficiency. Lancet Haematol. 2020; doi:10.1016/S2352-3026(19)30264-9.
26. Beguin Y, Aapro M, Ludwig H, Mizzen L, Osterborg A, et al. Epidemiological and nonclinical studies investigating effects of iron in carcinogenesis--a critical review. Crit Rev Oncol Hematol. 2014;89:1-15.
27. Keeler BD, Simpson JA, Ng O, Padmanabhan H, Brookes MJ, Acheson AG. Randomized clinical trial of preoperative oral versus intravenous iron in anaemic patients with colorectal cancer. Br J Surg. 2017;104:214-221.
28. Saito H. Metabolism of Iron Stores. Nagoya J Med Sci. 2014;76:235-254.
29. Saito H, Tomita A, Ohashi H, Maeda H, Hayashi H, Naoe T.et al. Determination of ferritin and hemosiderin iron in patients with normal iron stores and iron overload by serum ferritin kinetics. Nagoya J Med Sci. 2012;74:39-49.
30. Horniblow RD, Bedford M, Hollingworth R, Evans S, Sutton E, Lal N, et al. BRAF mutations are associated with increased iron regulatory protein-2 expression in colorectal tumorigenesis. Cancer

Sci. 2017;108:1135-1143.

31. Radulescu S, Brookes MJ, Salgueiro P, Ridgway RA, McGhee E, Anderson K, et al. Luminal iron levels govern intestinal tumorigenesis after Apc loss in vivo. Cell Rep. 2012;2:270-282.
32. Wu KJ, Polack A, Dalla-Favera R. Coordinated regulation of iron-controlling genes, H-ferritin and IRP2, by c-MYC. Science. 1999;283:676-679.
33. O'Donnell KA, Yu D, Zeller KI, Racke F, Thomas-Tikhonenko A, Dang CV. et al. Activation of transferrin receptor 1 by c-Myc enhances cellular proliferation and tumorigenesis. Mol Cell Biol. 2006;26:2373-2386.
34. Modjtahedi N, Frebourg T, Fossar N, Lavialle C, Cremisi C, Brison O. et al. Increased expression of cytokeratin and ferritin-H genes in tumorigenic clones of the SW 613-S human colon carcinoma cell line. Exp Cell Res. 1992;201:74-82.
35. Ng O, Keeler BD, Mishra A, Simpson JA, Neal K, Al-Hassi HO, et al. Iron therapy for preoperative anaemia. Cochrane Database Syst Rev. 2019; doi:10.1002/14651858.CD011588.

Figures

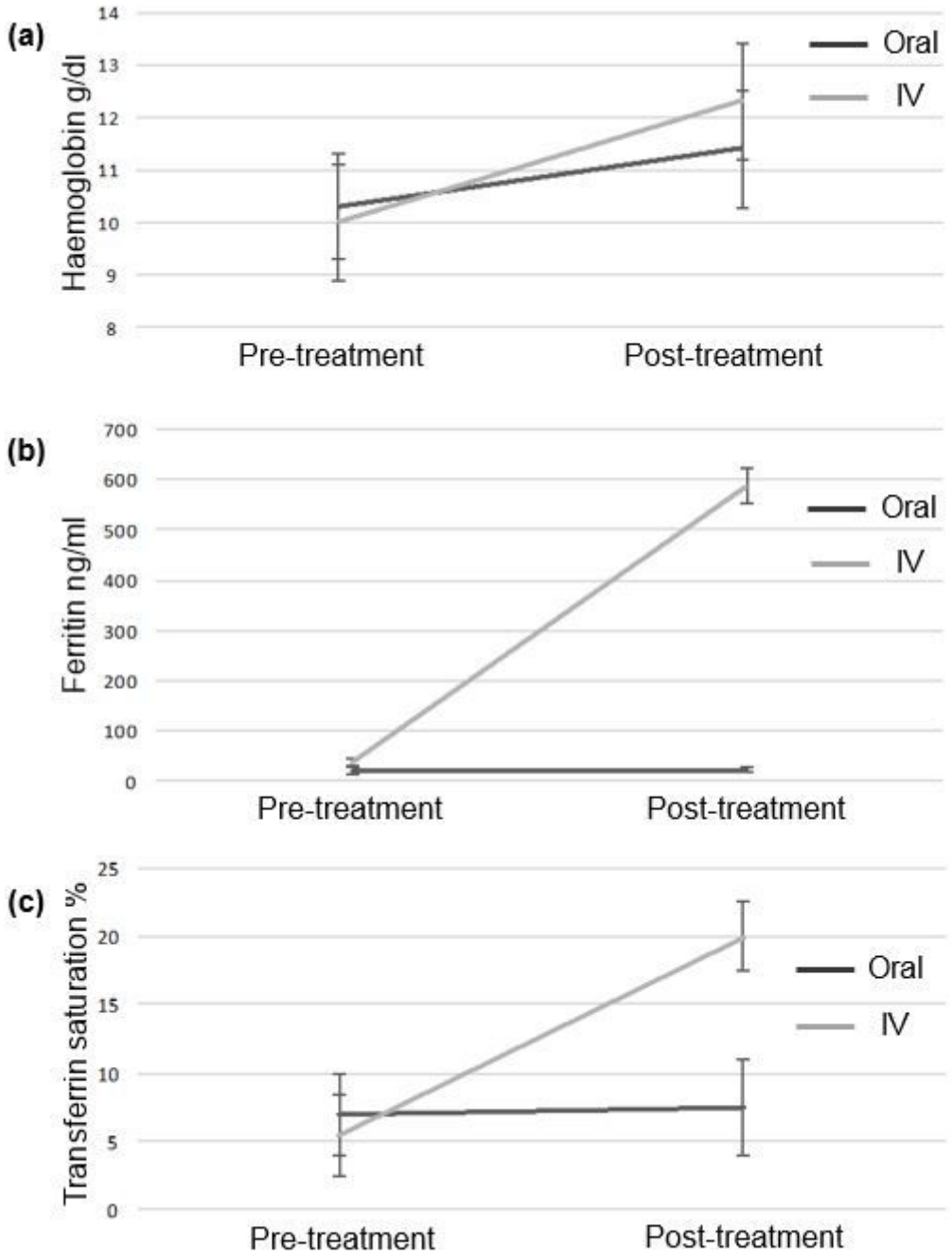

Figure 1

Clinical outcomes of included patients from the IVICA trial [citation] (a) Haemoglobin (b) Ferritin (c) Transferrin saturation

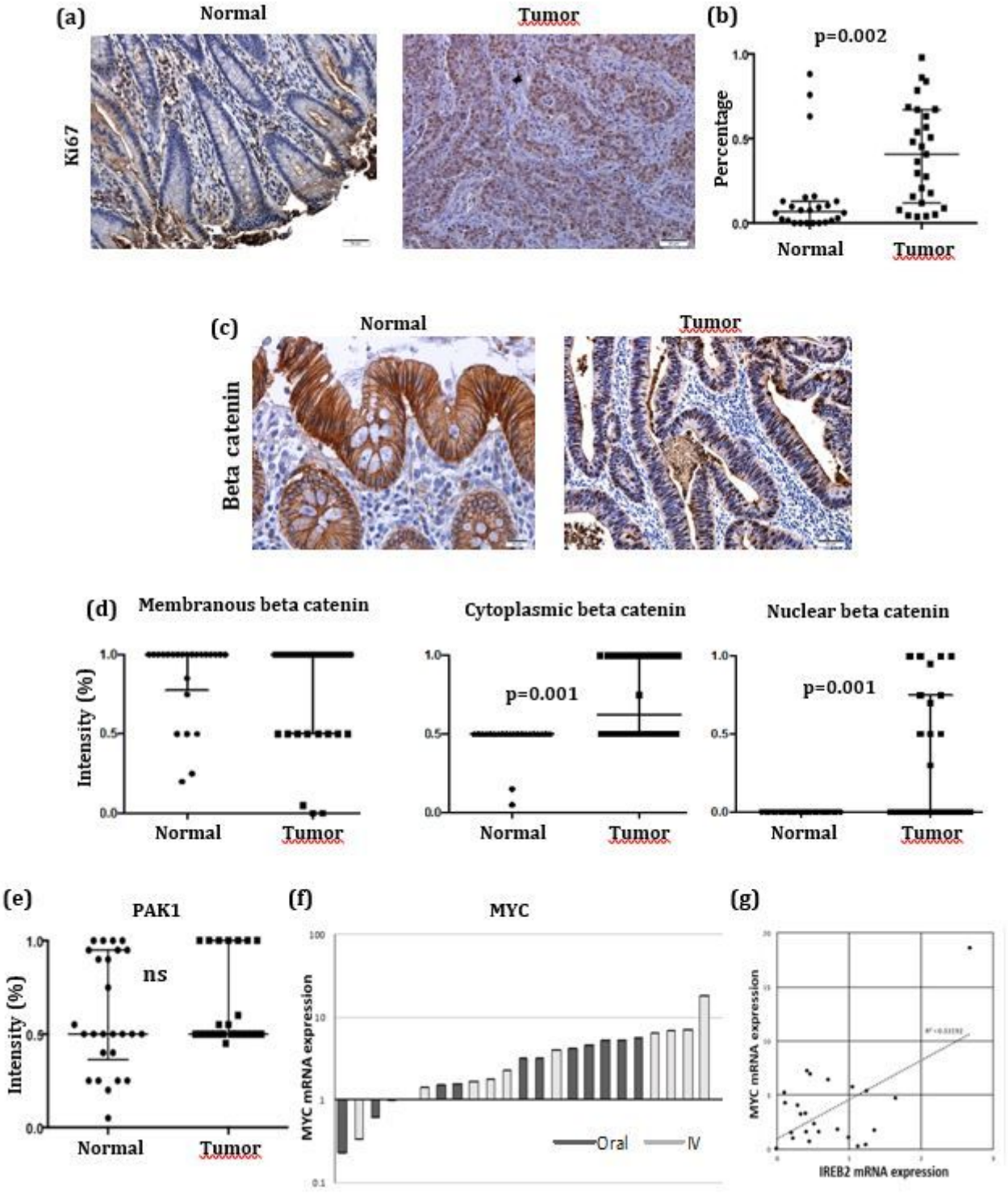

Figure 2

Immunohistochemistry photomicrographs and semi-quantitative analysis dot plots for proliferation markers in normal and tumor tissues from patients treated with oral or IV therapy. (a) Ki67 immunostaining; (b) analysis for PKi67 protein, dot plots with mean and standard deviation; (c) immunostaining of beta-catenin in normal and tumor tissues; (d) Dot plot analysis with mean and standard deviation of membranous, cytoplasmic and nuclear beta catenin expression; (e) Dot plot

analysis with mean and standard deviation of PAK1 immunostaining; (f) Real-time PCR fold change in c-MYC gene expression comparing oral versus intravenous iron groups; (g) IREB2 correlation with c-MYC scatter plots with regression line

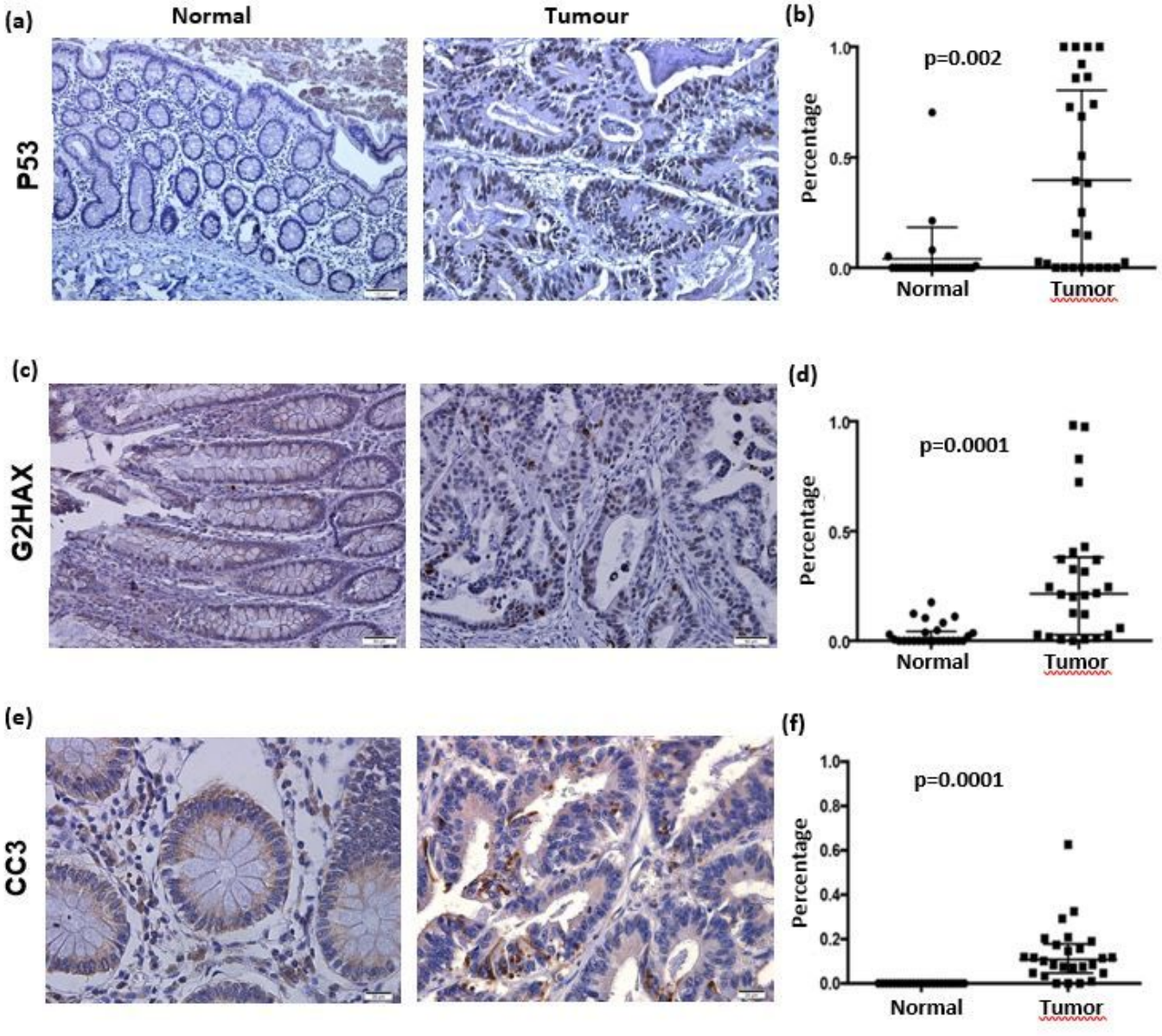

Figure 3

Immunohistochemistry photomicrographs and semi-quantitative analysis dot plots for apoptosis and DNA damage. (a) P53 immunostaining in normal and tumor tissues; (b) analysis for P53 protein, dot plots with mean and standard deviation; (c) γH2AX immunostaining in normal and tumor tissues; (d) analysis for γH2AX protein, dot plots with mean and standard deviation; (e) CC3 immunostaining in normal and tumor tissues; (f) analysis for CC3 protein, dot plots with mean and standard deviation

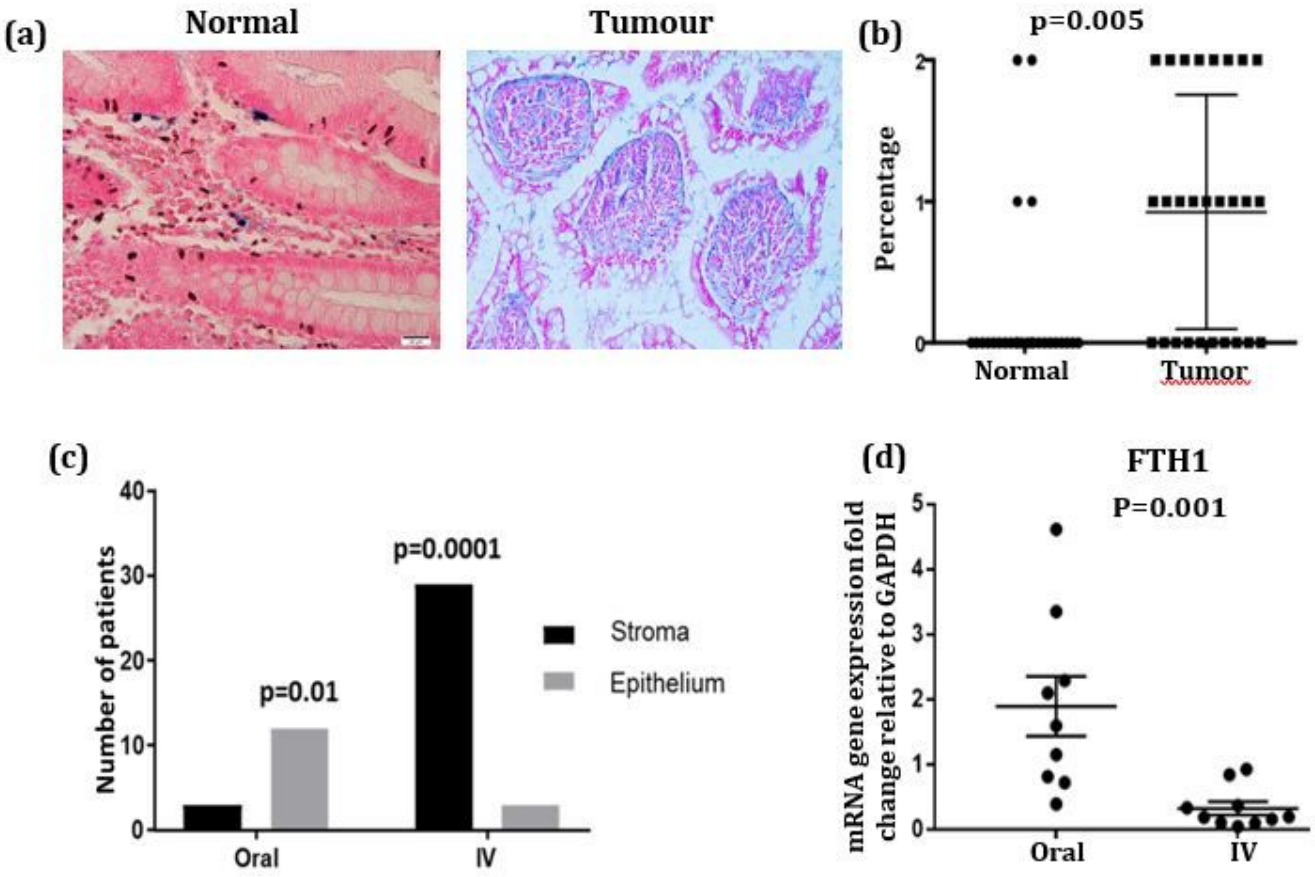

Figure 4

Perl's Prussian blue photomicrographs for iron loading. (a) Positive staining in normal and tumor cells; (b) analysis for Perl's Prussian blue, dot plots with mean and standard deviation; (c) Chi square analysis shown expression of Perl's Prussian blue in tumor and stroma of tumor tissue from patients treated with oral or IV iron. (d) Real-time PCR fold change in FTH1 gene expression comparing oral versus intravenous iron groups

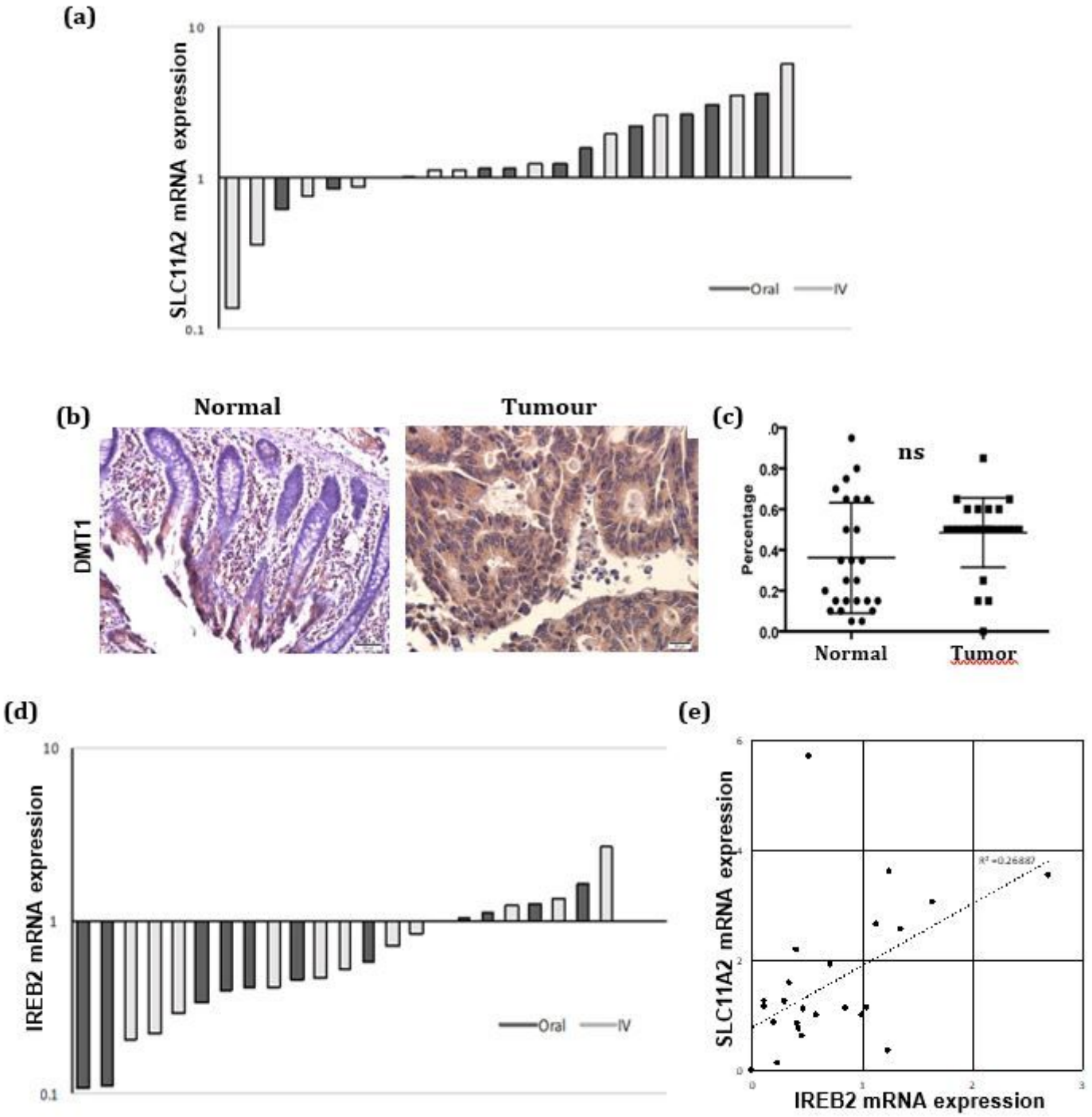

Figure 5

Real-time PCR fold change in gene expression and protein expression of cellular iron transport and iron regulation, comparing oral versus intravenous iron groups. (a) Real-time PCR fold change in SLC11A2 (DMT1) gene expression comparing oral versus intravenous iron groups. (b) Immunostaining of DMT1 in normal and tumor tissues. (c) Analysis for DMT1 protein, dot plots with mean and standard deviation. (d) Real-time PCR fold change in IREB2 gene expression comparing oral versus intravenous iron groups. (e) IREB correlation with DMT

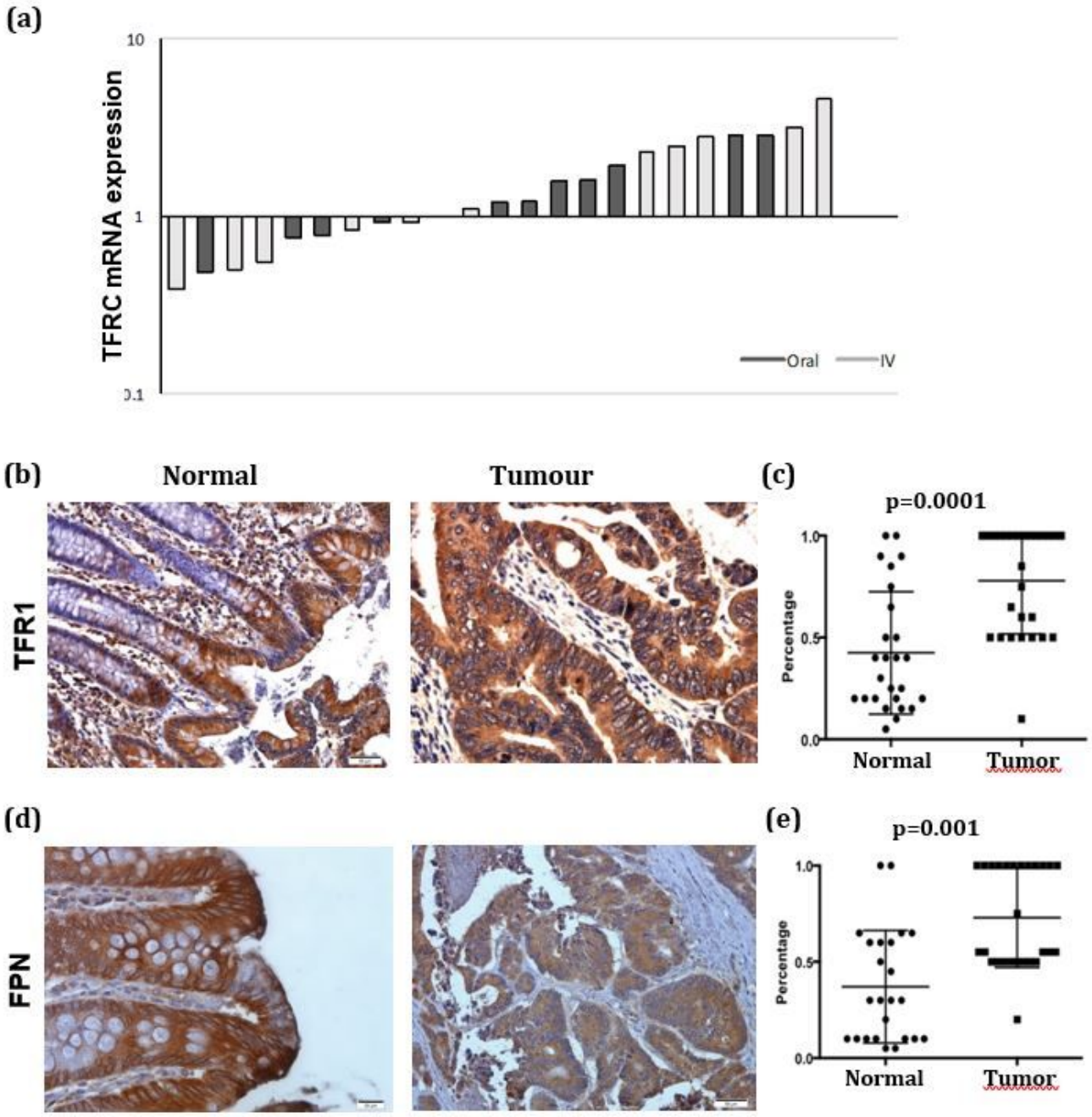

Figure 6

(a) Real-time PCR fold change in TFRC (TFR1) gene expression comparing oral versus intravenous iron groups. (b) immunostaining of TFR-1 in normal and tumor tissues. (c) Analysis for TfR1 protein, dot plots with mean and standard deviation. (d) immunostaining of ferroportin in normal and tumor tissues (e) analysis for ferroportin protein, dot plots with mean and standard deviation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalfileTable6.pdf
- AdditionalfileTable6.pdf
- AdditionalfileTable5.pdf
- AdditionalfileTable5.pdf
- AdditionalfilesTable4.pdf
- AdditionalfilesTable4.pdf
- AdditionalfileTable3.pdf
- AdditionalfileTable3.pdf
- AdditionalfileTable2.pdf
- AdditionalfileTable2.pdf
- AdditionalfileTable1.pdf
- AdditionalfileTable1.pdf