Peer Review File

Article Information: Available at http://dx.doi.org/10.21037/tlcr-20-341.

Replies to Reviewer A:

Comment 1: The abstract was too simple. Please enrich the progress of the role of ferrous in the abstraction.

Reply 1: We thank Reviewer A for this comment and thank you very much for your careful reading! We have enriched the progress of the role of ferrous in the abstraction according to your suggestion (see Page 4, lines 54-57). We also supplemented the key contents of our manuscript (see Pages 4-5, lines 50-67).

Changes in the text: In the abstraction, we have enriched the progress of the role of ferrous: "Excessive iron from aberrant iron metabolisms or the maladjustment of the two main redox systems thiols and lipid peroxidation role as the major causes of ROS generation, and the redox-acrive ferrous (intracellular labile iron) is a crucial factor for the lipid peroxidation" (see Page 4, lines 54-57). The abstract was modified to "Ferroptosis is a novel form of non-apoptotic regulated cell death (RCD), with distinct characteristics and functions in physical conditions and multiple diseases such as cancers. …… Here, we summarize the ferroptosis characters from its underlying basis and role in cancer, followed by its possible applications in cancer therapies and challenges maintained" (see Pages 4-5, lines 50-67)."

Comment 2: In the introduction, summary briefly the progress about ferroptosis in other disease except tumor.

Reply 2: Thank you for this comment. We have summarized the progress about ferroptosis in other disease except tumor briefly and added it in our manuscript (see Page 7, lines 98-104).

Changes in the text: In the introduction, we have added the progress about ferroptosis in other disease except tumor briefly: "Ferroptosis induction is associated with multiple disease occurrence, including immune system (nonalcoholic steatohepatitis), brain (stroke and intracerebral hemorrhage),

neurodegenerative (Alzheimer's disease (AD), Huntington's disease (HD), and Parkinson's disease (PD)), heart (heart failure), and blood diseases (leukemia) (7,12). Inhibiting ferroptosis has been identified as a potential prevention or therapeutic strategies for some of these diseases" (see Page 7, lines 98-104).

Comment 3: Please make clear about the difference between apoptosis and ferroptosis in the introduction.

Reply 3: This is an important point and we thank you for this comment. We certainly agree that it is necessary to make clear about the difference between apoptosis and ferroptosis. Therefore, we added a table named "Table.1 Cell morphology, biochemical features, and key regulators of ferroptosis, apoptosis, necroptosis" to discuss the difference between apoptosis and ferroptosis (see Table.1), which matched the sentence in text (see Pages 6-7, lines 96-98).

Changes in the text: We have added the sentences "Unlike apoptosis and autophagy, ferroptosis is iron-dependent, with specific characteristics of cytological changes such as the rupture of outer mitochondrial membrane, small mitochondria with the condensed mitochondrial membrane, and a vanishing or decrease of mitochondria cristae (6-8). Based on the original studies of cell death, ferroptosis is markedly different from the other RCD types such as apoptosis and autophagy at levels of cell morphology, biochemical features, and regulations (Table.1) (9-11)" in our text (see Pages 6-7, lines 96-98). In addition, we added a table named "Table.1 Cell morphology, biochemical features, and key regulators of ferroptosis, apoptosis, necroptosis" to discuss the difference between apoptosis and ferroptosis" (see Table.1).

Table.1 Cell morphology, biochemical features, and key regulators of ferroptosis, apoptosis, necroptosis

Type of cell death	Cell morphology	Biochemical features	Key regulators
Ferroptosis	Small mitochondria with a condensed mitochondrial membrane, vanishing or	Iron loading, ROS accumulation, System X_c^- inhibition with reduced	Positive: p53, Ras, VDAC2/3, TFR1, NOX; Negative: SLC7A11,

	reduction of mitochondria crista, and rupture of outer mitochondrial membrane.	GSH, GPX4 inhibition	GPX4, NRF2, HSPB1
Apoptosis	Plasma membrane blebbing; reduction of cellular and nuclear volume; nuclear fragmentation; and chromatin condensation	Activation of caspases and proapoptotic Bcl-2 family proteins, Oligonucleosomal DNA fragmentation, exposure of Plasma membrane rupture, dissipation of dissipation	Positive: pro-apoptotic Bcl-2 family proteins (Bax, Bak), p53; Negative: anti-apoptotic Bcl-2 family proteins (Bcl-2, Bcl-XL)
Autophagy	(Double-membraned) autolysosome accumulation, Cytoplasmic vacuolization	Conversion from LC3-I to LC3-II, degradation of p62Lck, Beclin-1 dissociation from Bcl-2/XL	Positive: Beclin 1, ATG family proteins (ATG5, ATG7)

Comment 4: How about the changes of cell morphology in apoptosis, autophagy and ferroptosis.

Reply 4: Thank you for this suggestion. We have enriched the changes of cell morphology in apoptosis, autophagy and ferroptosis in our manuscript (see Page, lines) (see Table.1).

Changes in the text: We have added this "Unlike apoptosis and autophagy, ferroptosis is iron-dependent, with specific characteristics of cytological changes …… ferroptosis is markedly different from the other RCD types such as apoptosis and autophagy at levels of cell morphology, biochemical features, and regulations (Table.1) (9-11)" in the introduction, (see Pages 6-7, lines 92-98). We also made a table about this changes (see Table.1).

Comment 5: Please enrich the correlation between ferrous metabolism and tumor in the review.

Reply 5: **Thank you for this comment, and we have enriched the correlation between ferrous metabolism and tumor in our manuscript (see Pages 8-9, lines 118-135).**

Changes in the text: **We have modified and enrich this correlation into: "The most prominent character of ferroptosis is ROS generation, mainly caused by iron metabolism disorders (7). The endosomal uptake of circulated iron (Fe3+) is mediated by its binding to transferrin (TF) and transferrin receptor 1 (TFR1). Iron Fe3+ is deoxidized to iron Fe2+, under the catalysis of iron oxide reductase named six-transmembrane epithelial antigen of the prostate 3 (STEAP3), and ultimately release into labile iron pool (LIP), due to Fe2+'s characteristics of high solubility and transfer electron capability. Increasing formation of LIP may trigger the Fenton reaction (the process of ROS generation mediated by interaction between Fe2+ and hydrogen peroxide (H2O2)), which may result in iron poisonous (16). Compared with RAS un-mutated ferropptosis-insensitive cells, RAS-mutated ferroptosis-sensitive cells increased the expression of TFR1 and decreased the expression of ferritin light chain (FTL) and ferritin heavy chain 1 (FTH1) in the iron-storage protein subunits. This suggests that increasing iron intake and reducing iron storage may cause iron overload, in the end, leading to ferroptosis (17). Thus, intracellular iron metabolism homeostasis regulates ferroptosis process. Cancer cells become more vulnerable to iron toxicosis and ROS accumulation than noncancerous cells with a powerful iron dependency characteristic, making it possible for application of ferroptosis inducers in cancer therapy (18,19)" (see Pages 8-9, lines 118-135).**

Comment 6: What is the meaning of "TME"? How about the role of hepcidin-ferroportin in ferroptosis? How about the correlation miRNAs in ferroptosis?

Reply 6: **The "TME" in our manuscript means "tumor microenvironment" and we have made it clear in our manuscript (see Page 13, lines 199-200). This comment is much appreciated and thank you very much for the helpful suggestions. We are very interested in and fully agree the excellent suggestions of Reviewer A and therefore we conducted a further study about the role of hepcidin-ferroportin and related miRNAs in ferroptosis respectively. We recognize this is**

an important point and therefore, we have added two parts to analyze these correlations in ferroptosis (see Pages 15-16, lines 225-238), (see Page 16, lines 239-251).

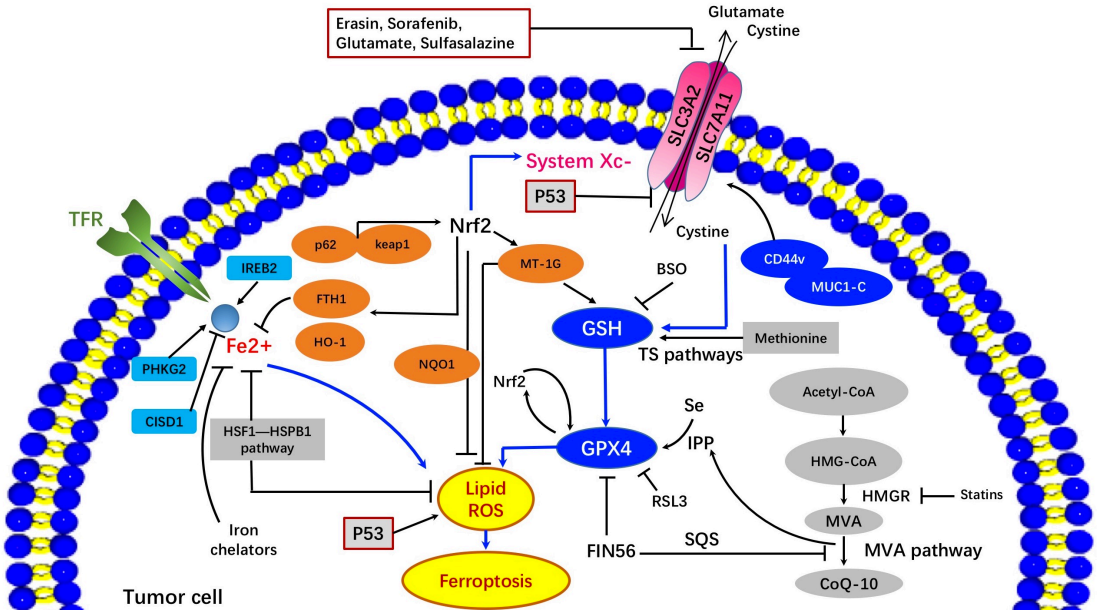
Changes in the text: We have added the meaning of "TME" in our text: tumor microenvironment (TME) (see Page 13, lines 199-200). In addition, two parts named "2.4 Hepcidin-ferroportin in ferroptosis of cancers" and "2.5 MicroRNAs in ferroptosis of cancers" were added. Part 2.4: "Hepcidin and FPN1 constitute the vital regulator of the systemic iron homeostasis (38). As the negative regulator of protein FPN-1, hepcidin inhibits the release of iron from macrophages and intestinal mucosal cells into circulation…… Pancreatic cancer cells treated with ruscogenin were also observed elevated intracellular iron, ROS generation, and ferroptosis by downregulating FPN-1 and upregulating TF (52). Thus, regulator hepcidin-ferroportin plays an important role in iron homeostasis and ferroptosis in cancers" (see Pages 15-16, lines 225-238). Part 2.5: "MicroRNAs (miRNAs) are a class of small non-coding RNA molecules, which can regulate the posttranscriptional gene expression…… The miR-4715-3p (56), miR-103a-3p (57), and miR-6852 (58) were also identified as regulators in ferroptosis in upper gastrointestinal, gastric, and lung cancers respectively. Thus, miRNAs play important roles in iron metabolisms and ferroptosis" (see Page 16, lines 239-251).

Comment 7: Please summary ferroptosis modulation in tumor in a figure.

Reply 7: This is an excellent suggestion, and we thank Reviewer A for bringing it up. We have added a figure named "Figure.2 Ferroptosis modulation in tumor" (see Fiure.2). We also added its legend in this review (see Pages 37-38, lines 701-722).

Changes in the text:

The word version of figure.2:



The figure legend: "Figure.2 Ferroptosis modulation in tumor

Small molecules such as erasin, sorafenib, glutamate, and sulfasalazine induce ferroptosis by inhibiting system Xc- and impeding cysteine uptake, which could result in a subsequent decline of glutathione and a decrease of cells' anti-oxidative ability. mucin 1 C-terminal (MUC1-C) binds with CD44v to promote stability of the system Xc-. The cysteine level can also be supplemented by cellular methionine *via* the sulphur-transfer pathways. GPX4 can prevent ferrpotosis by suppressing cellular lipid peroxides and the mevalonate (MVA) pathway is crucial for its maturation and the products of it (IPP and CoQ10) can promote synthesis of GPX4. Treatment FIN56 modulates squalene synthase (SQS) to reduce CoQ10. Ferroptosis inducer RSL3 can suppress GPX4 directly to regulate ferroptosis. The p62-Keap1-Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway is able to regulate Nrf2-targeted genes such as heme oxygenase-1 (HO-1), ferritin heavy chain 1 (FTH1), and NAD(P)H: quinone oxidoreductase 1 (NQO1) against ferroptosis. CISD1, PHKG2, and IREB2 are important in regulating iron metabolism and ferroptosis. Ironchelators can inhibit ferroptosis. The HSPB1 also impedes ferroptosis by inhibiting increase of intracellular iron. In addition, p53 also regulate ferroptosis through inhibiting SLC7A11 and promoting lipid peroxides production. Abbreviations: BSO, buthionine sulfoximine; FTH1, ferritin heavy chain 1; HSP, heat-shock protein; HO-1, heme oxygenase-1; MUC1-C, mucin 1 C-terminal; MVA, mevalonate; NQO1, NAD(P)H: quinone oxidoreductase 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; SQS, squalene

synthase" (see Pages 37-38, lines 701-722).

Comment 8: Are there any activators induced ferroptosis? Please supplement in the review.

Reply 8: Yes. We have carefully checked the reports related to ferroptosis inducers and found some activators to induce ferroptosis. Thank you for this suggestions, and we have supplemented it in the review (see Pages 18-19, lines 280-292). We also added the related molecules in Table.2 (see Table.2).

Changes in the text: We have added a paragraph in this review: "Beyond inhibition of system Xc- and GPX4, ferroptosis can also be induced by increasing LIP. For example, molecular BAY 11-7085 could induce ferroptosis through the Nrf2-SLC7A11-HO-1 pathway, and the overexpression of heme oxygenase-1 (HO-1) that is encoded by HMOX1 has been observed in MDA-MB-231 breast cancer cells and DBTRG-05MG glioblastoma cells (64). Increased TF expression and decreased FPN-1 expression could also mediate ferroptosis using siramesine and lapatinib (65). In addition, autophagy also contributes to inducing ferroptosis by degradating ferritin in cancer cells. The cargo receptor nuclear receptor coactivator 4 (NCOA4) is important in the autophagic turnover of the ferritin in ferroptosis. In pancreatic cancer cells, overexpression of NCOA4 *via* gene transfection inhibited FIH1 expression and promoted erasein-induced ferroptosis (66). Further studies are needed to explore novel molecules targeting NCOA4 in ferroptosis to treat cancer. Beyond these small molecules, more are involved in inducing ferroptosis (Table.2)" (see Pages 18-19, lines 280-292). We also added the related molecules in Table.2. The content that is not modified has not been shown here.

Reagents	Mechanisms	Formula	*in-vitro*	*in-vivo*	Refs

BAY 11-7085	Increase in LIP by HMOX1 upregulation	C13H15NO2S	MCF-7, MDAMB-468, MDA-MB-231, A549, SKBR3, SKOV3	NA	(64)
Bromelain	upregulation of / ACSL-4 in Kras mutant CRC cells		CT-116, DLD-1	NA	(90)

Replies to Editorial Comments:

Many thanks to editors for your comments!

Comment 1: Please provide the Conflict of Interest Form suggested by ICMJE (http://www.icmje.org/conflicts-of-interest/). **Each author should submit a separate form** and is responsible for the accuracy and completeness of the submitted information.

(1) Download the attached form to your computer.

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(3) **Collect all authors' COI forms, and number them in the same order with the appearance of names in title page. Then submit.**

Reply 1: Thank you for this comment! We have downloaded, filled out, and collected all COI forms. We also numbered them in the same order with the appearance of names in title page and submitted them.

Comment 2: Please indicate if any of the authors serves as an Editorial Board Member or Section Editor for this journal. State "*None*" in the reply if it's otherwise.

Reply 2: The corresponding author Dr. Caicun Zhou serves as an Editorial Board Member of this journal.

Comment 3: We suggest making the abstract more informative which should be no less than 200 and no more than 350 words.

Reply 3: We have enriched the key contents in the abstract to make it more informative, and the word count is 212, which meets the requirements. Thank you very much for this comment. Now the abstract is: "Ferroptosis is a novel form of non-apoptotic regulated cell death (RCD), with distinct characteristics and functions in physical conditions and multiple diseases such as cancers. Unlike apoptosis and autophagy, this new RCD is an iron-dependent cell death with features of lethal accumulation of reactive oxygen species (ROS) and over production of lipid peroxidation. Excessive iron from aberrant iron metabolisms or the maladjustment of the two main redox systems thiols and lipid peroxidation role as the major causes of ROS generation, and the redox-acrive ferrous (intracellular labile iron) is a crucial factor for the lipid peroxidation. Regulation of ferrroptosis also involves different pathways such as mevalonate pathway, P53 pathway and p62-Keap1-Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway. Ferroptosis roles as a double-edged sword either suppressing or promoting tumor progression with the release of multiple signaling molecules in the tumor microenvironment. Emerging evidence suggests ferroptosis as a potential target for cancer therapy and ferroptosis inducers including small molecules and nanomaterials have been developed. The application of ferroptosis inducers also relates to overcoming drug resistance and preventing tumor metastasis, and may become a promising strategy combined with other anti-cancer therapies. Here, we summarize the ferroptosis characters from its underlying basis and role in cancer, followed by its possible applications in cancer therapies and challenges maintained" (see Abstract).

Comment 4: Please revise and rearrange the main text into IMRaD structure: **Introduction, Methods, Results, and Discussion.**

Reply 4: Since the type of our manuscript is review, we have retained the original format. If there are any problems in our revised manuscript, please do not hesitate to contact us. We will try our best to revise the paper more.

Comment 5: Please add ethical statement into the manuscript. - Ethical Statement (This statement must be included in the footnote) - *the authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved*.

Reply 5: Thank you for your kind reminder. We have added the "Ethical Statement- ***the authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved*****" in the footnote of our manuscript.**

Comment 6: The wordings of the main text and/or figures/tables should be checked by a native English-speaking expert who is majoring in your field. We suggest that you consult a professional language checker (e.g., AME Editing Service).

Reply 6: This is an important point and we thank you for this comment. We have carefully considered the comments and have revised the manuscript accordingly. We also asked two native English speakers to help us and polished the expression of our manuscript.

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