Peer Review File

Article information: http://dx.doi.org/10.21037/tcr-22-992

Review Comments

The article titled as “Development and validation of a combined ferroptosis and immune prognostic signature for lung adenocarcinoma” was quite interesting. The authors used bioinformatics and public datasets for elucidating the interaction between ferropotiss and immunity. It would be worthy of publishing if the authors could have more clearly explanations for your article.

Major

Comments 1:

The concept of “Generation of CSFI”: how to generate? The authors should clarify and persuade the readers and the reviewers.

Reply 1:

Thank you very much for your insightful comment and suggestion, which markedly contributed to the improvement of our manuscript. CSFI is an important concept throughout our article.

In our study, CSFI, the abbreviation of combined signature of ferroptosis and immune-related genes, was composed of several ferroptosis-related genes (FRGs) or immune-related genes (IRGs). **The screening process for these genes was as follows. Firstly,** differentially expressed genes (DEGs) between tumor samples and normal samples were identified with the absolute values of $|\log FC| \geqslant 1$ and FDR <0.05. Overlapping genes of these DEGs with FRGs and IRGs were obtained separately. **Secondly,** these overlapping genes, that is, differentially expressed FRGs and IRGs were used for univariate Cox regression to identify prognostic genes. FRGs with $P<0.05$ and IRGs with $P<0.01$ were regarded as prognostic genes. **Thirdly,** these prognostic genes were used to construct the LASSO-Cox regression model. Finally, coefficients and expression levels of 8 genes were used to calculate CSFI values in LUAD patients. CSFI = (0.0052*ANGPTL4 exp.) + (-0.0260*ARRB1 exp.) + (0.0030*CAV1 exp.) + (0.0063*CXCL5 exp.) + (-0.0003*HLA-DRA exp.) + (-0.0135*IL33 exp.) + (0.0052*INHA exp.) + (-0.0469*LIFR exp.).

Changes in the text:

We sincerely apologize for our ambiguous expression about CSFI.

First of all, according to the generation of CSFI, we have modified our manuscript in “Materials and Methods” section as advised (see Page 7, line 6-21). Meanwhile, to make the process of generating CSFI clearer, we have divided the original "Generation of CSFI" in “Materials and Methods” section into two parts, namely “Generation of CSFI” and “Clinical Benefit Assessment”, and modified our flow diagram (*Figure 1*).

Comments 2:
What are the meanings of “high” and “low” CSFI in ferroptosis and immunity?

Reply 2:
Thank you very much for your comment. In this study, LUAD patients from TCGA were divided into CSFI-high and CSFI-low groups based on the median CSFI values. Then, we assessed ferroptosis and immune status of LUAD patients with high- and low-CSFI values to guide dual-target therapy in LUAD patients.
On the one hand, LUAD patients with high CSFI values appeared ferroptosis inhibition state. The upregulation of suppressors of ferroptosis, including HSF1, HSPB1, NQO1, and SLC7A11, represented that patients from the CSFI-high group might be equipped with strong ferroptosis resistance and the function of ferroptosis inducers might be diminished. Therefore, the therapy by combining ferroptosis inducers and targeting SLC7A11, HSF1, and HSPB1 might result in better outcomes in the CSFI-high group.
On the other hand, LUAD patients with high CSFI values appeared immune-suppressive state. It is possible that immunotherapy is less effective in high-risk groups than in low-risk groups.
Therefore, based on our findings, we believe that a synergistic treatment of immunity and ferroptosis would be more effective for LUAD patients with low CSFI values. This provides ideas for the clinical treatment of LUAD patients.

Changes in the text:
We have strengthened the description in the discussion section (see Page 14, line 16-22, Page 15, line 1-22, Page 16, line 1-22, Page 17, line 1-12).

Comments 3:
Based on the abstract, I could not figure out the significance of CSFI in lung adenocarcinoma.

Reply 3:
Thank you very much for your comment. Firstly, the CSFI constructed in this study could predict the prognoses of LUAD patients robustly. Patients with high CSFI values showed better prognoses than those with low CSFI values. In addition, CSFI which served as a classifier divided LUAD patients into ferroptosis^{low}/immunity^{low} and ferroptosis^{high}/immunity^{high} groups. And the ferroptosis and immune statuses of LUAD patients could contribute to guide molecularly targeted therapies.
Therefore, based on our findings, we believe that the CSFI could predict prognoses and contribute to guiding LUAD patients with personalized targeted therapy.
Changes in the text:
We sincerely apologize for our ambiguous expression about the significance of CSFI in the abstract section**.** We added some data in the abstract. (see Page 3, line 7-11)

Comments 4:
Usually, most the study are investigating “a gene” disease but not “a group of genes” disease.

Reply 4:
Thank you very much for your insightful comment and suggestion, which markedly contributed to the improvement of our manuscript. In recent years, there has been increasing evidence that variations in patient genomes contribute significantly to interpatient differences in drug response (1). At the same drug dosage, a certain group of patients may experience no therapeutic benefit, whereas others may develop severe adverse drug reactions. Therefore, an increasing number of studies have attempted to identify subgroups of tumor patients based on their molecular profiles. These subgroups possess unique phenotypes, different treatment responses, and different prognoses. In the past decade, with the rapid development of high-throughput sequencing, gene set has shown great potential in personalized therapy. Specific gene set has led to a better understanding of the biological phenotypes of cancer than single biomarkers (2).

For example, in one study, seven pyroptosis-related genes were used to classify patients with ovarian cancer into high- and low-risk groups. And ultimately it was found that patients in the high-risk group had a state of immunosuppression(3). Moreover, hypoxia-immune signature which was composed of ten hypoxia-related or immune-related genes divided patients with triple-negative breast cancer into two subgroups. It was proved that immunotherapy may be more effective in patients in the hypoxia low/immune high group (4).

References

1. Lee JW, Aminkeng F, Bhavsar AP, et al. The emerging era of pharmacogenomics: current successes, future potential, and challenges. Clin Genet 2014;86(1):21-8.
2. Tsimberidou AM, Fountzilas E, Nikanjam M, et al. Review of precision cancer medicine: Evolution of the treatment paradigm. Cancer Treat Rev 2020;86:102019.
3. Ye Y, Dai Q, Qi H. A novel defined pyroptosis-related gene signature for predicting the prognosis of ovarian cancer. Cell Death Discov 2021;7(1):71.
4. Zheng S, Zou Y, Liang JY, et al. Identification and validation of a combined hypoxia and immune index for triple-negative breast cancer. Mol Oncol 2020;14(11):2814-33.

Changes in the text

We have mentioned in our text (see Page 13, line 19-22, Page 14, line 1-7). We also added some data in the introduction section (see Page 4, line 5-14).

Comments 5:
In this article, I could not know the meaning of ferroptosis in lung cancer.

Reply 5:
Thank you for pointing this out and we are happy to follow.
Ferroptosis, discovered in 2012, was first identified as a type of oxidative iron-dependent programmed cell death (PCD) different from apoptosis, necrosis, and autophagy. Ferroptosis elicits a therapeutic response that inhibits tumor growth when used experimental reagents (e.g., erastin and RSL3), approved drugs (e.g., sulfasalazine and artemisinin), ionizing radiation, and cytokines (e.g., IFNγ and TGFβ1) (1). Studies have shown that dysregulation of ferroptosis may contribute to lung cancer development (2). Numerous studies have explored the relationship between certain ferroptosis-related genes and lung cancer. Lai et al. (3) found that glutathione peroxidase 4 (GPX4) overexpressed in NSCLC (non-small cell lung cancer) tissues and cell lines, and the upregulation of GPX4 reduced multiple mitochondrial abnormalities which is one of the features of ferroptosis and promoted proliferation of lung cancer cells. Moreover, cystine/glutamate antiporter (SLC7A11), regulating metabolic demand during the development of NSCLC, was upregulated in NSCLC tissues and was associated with a poorer 5-year survival rate (4). Huang et al. (5) found that in LUAD cells (A549 cells), p53 activation by erastin exposure induced ferroptosis and apoptosis and arrested the cell cycle at the G1 phase, thereby inhibited cell proliferation. **For LUAD, targeting ferroptosis may be beneficial to explore more effective anticancer therapies.**

Abnormal ferroptosis is closely associated with a dysregulated immune response. The interactions among ferroptosis, inflammation, and the immune system in the tumor microenvironment (TME) influence tumor progression. An important study found that activation of CD8+ T cells by immunotherapy resulted in the release of interferon-γ to downregulate the expression of SLC3A2 and SLC7A11, which in turn enhanced ferroptosis-specific lipid peroxidation (6). The synergy of ferroptosis and immunity not only inhibits primary tumor but also stimulates immune responses by combining with immune checkpoint inhibitors (ICIs) (7). **In conclusion, exploring the integrated regulatory network of ferroptosis and immunity in LUAD might provide clues to new anti-cancer strategies.**

Therefore, in our study, we used the combined signature of ferroptosis and immune-related genes (CSFI) to distinguish different subgroups, and then assessed the ferroptosis and immune status of LUAD patients with high- and low-CSFI values to guide personalized therapy.

References
1. Tabnak P, HajiEsmailPoor Z, Soraneh S. Ferroptosis in Lung Cancer: From Molecular Mechanisms to Prognostic and Therapeutic Opportunities. Front Oncol. 2021;11:792827.

2. Li G, Yang J, Zhao G, et al. Dysregulation of ferroptosis may involve in the development of non-small-cell lung cancer in Xuanwei area. J Cell Mol Med 2021;25(6):2872-84.
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5. Huang C, Yang M, Deng J, et al. Upregulation and activation of p53 by erastin-induced reactive oxygen species contribute to cytotoxic and cytostatic effects in A549 lung cancer cells. Oncol Rep 2018;40(4):2363-70.
6. Wang W, Green M, Choi JE, et al. CD8(+) T cells regulate tumour ferroptosis during cancer immunotherapy. Nature 2019;569(7755):270-4.
7. Li Z, Rong L. Cascade reaction-mediated efficient ferroptosis synergizes with immunomodulation for high-performance cancer therapy. Biomater Sci 2020;8(22):6272-85.

Changes in the text

We described these details in the Introduction (see Page 4, line 15-22, Page 5, line 1-22, Page 6, line 1-4).

Comments 6:

Please have someone who is mastering in the scientific writing and grammar/wording.

Reply 6:

Thank you very much for your insightful comment and suggestion, which markedly contributed to the improvement of our manuscript. Following your comment, the authors had also involved native English speakers for language corrections.

Changes in the text

All of the changes in English language writing and grammar were highlighted in green color in the revised manuscript.