Ferroptosis gene signature in cholangiocarcinoma

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Ferroptosis is a recently discovered iron-dependent form of cell death that is distinct from apoptosis, necrosis, and autophagy. Many studies suggest that ferroptosis plays a critical role in tumor suppression, opening up new avenues for cancer therapy. In this issue of *Molecular Therapy – Oncolytics*, Wenchao Yao et al.[1] examined the role of ferroptosis in cholangiocarcinoma (CCA or CHOL) and its association with the tumor microenvironment by analyzing single-cell RNA sequencing (scRNA-seq) data in the Gene Expression Omnibus (GEO) database and integrating it with clinical data from patients with CCA. These findings suggest that the communication between monocytes and malignant cells in CCA may regulate ferroptosis in cancer cells and that a three-gene signature associated with monocytes and ferroptosis can be used to accurately predict the prognosis risk for CCA.

CCA is a malignant invasive carcinoma originating from cholangiocytes. CCA has an insidious beginning, is extremely invasive, and can affect perihepatic tissues and lymph nodes.[2] Most patients with CCA are advanced when diagnosed, and despite therapeutic choices, their prognosis is bleak.[3] Therefore, early molecular diagnosis and prognosis of CCA improve patient survival. Ferroptosis is a type of programmed cell death caused by Fe-dependent lipid peroxidation and is morphologically, biochemically, and genetically distinct from apoptosis, necrosis, and autophagy. Ferroptosis plays an important role in inhibiting tumor cell proliferation, invasion, and metastasis. Ferroptosis can cause an immunological response in tumors, especially in malignancies that are resistant to standard treatments, and ferroptotic tumor cells secrete signaling molecules that can either inhibit or promote tumor growth.[4] The role of ferroptosis in a variety of cancers is now being studied, although the role of these ferroptosis molecules in CCA remains unknown. Therefore, it is essential to investigate ferroptosis' potential applications to CCA. Single-cell sequencing can be used to investigate tumors from a variety of perspectives including tumor heterogeneity, the tumor microenvironment, cancer cell metastasis and spread, and the development of drug resistance in cancer cells.[5] CCA is an aggressive cancer that is resistant to chemotherapy. It is caused by the different types of immune cells, stromal cells, and malignant cells in the tumor microenvironment. Therefore, a comprehensive understanding of the complex transcriptional architecture and intercellular crosstalk is essential to elucidate the mechanisms driving CCA progression and the development of more effective therapies. In this issue, Wenchao Yao et al.[1] investigated scRNA-seq data in the GEO database by combining the bulk data in The Cancer Genome Atlas (TCGA) and GEO with the clinical information of patients with CCA.

In order to evaluate the role of diverse immune cell infiltrates in CCA, Wenchao Yao et al.[1] used bulk RNA-seq data and investigated the proportion of immune cells and their effect on the prognosis of CCA. The scRNA-seq dataset (GEO: GSE138709) was utilized to characterize the diversity and heterogeneity of different cell subsets in CCA tissue. CIBERSORT was used to look at immune cell infiltration in this study, and it was found that monocyte infiltration was linked to a better prognosis. To investigate the effect of monocyte infiltration on CCA cells, an intercellular communication analysis was performed between cholangiocytes and monocytes in CCA, and it was observed that the association between TNFSF13B and TFRC was significant. TNFSF13B is a monocyte cytokine marker, and TFRC is a ferroptosis driver and marker that controls ferroptosis by acting as a channel for Fe3+ in the outer membrane.[6] Compared with benign epithelial cells, malignant epithelial cells were considerably enriched in ferroptosis signaling pathways, and TFRC was significantly overexpressed in the cluster 4 subset. Intriguingly, it also found that the mineral absorption signaling pathway was considerably enriched and that the enriched genes (FTH1, FTL) in the system were strongly associated with Fe3+ transport. They hypothesize that monocyte-derived TNFSF13B facilitates the transport of external Fe3+ by binding to the TFRC receptor on the surface of cluster 4 and consequently inducing the ferroptosis process to occur. Using univariate and multivariate Cox regression analysis, a prognostic signature comprising three genes (BNIP3, TMEM107, and CENPW) was constructed. The accuracy of the signature in predicting the prognosis of CCA was further evaluated using receiver operating characteristic (ROC) and decision curve analysis (DCA) curves. In addition, they developed a nomogram risk assessment map that incorporated risk score and clinical parameters to enable clinical application. Over the past few years, there has been more and more evidence that BNIP3, TMEM107, and CENPW could be targets for CCA. Also, they started by looking at how immune cell infiltration affected the prognosis of CCA, and then they used single-cell sequencing to look at the data and find a possible way that monocytes could control malignant intracellular ferroptosis. This study not only expands our understanding of ferroptosis in cancer cells in the tumor microenvironment but also gives the combined TCGA-CCA and FerrDb databases a ferroptosis-monocyte signature. In conclusion, they demonstrated that ferroptosis in cancer cells may be regulated by the communication between monocytes and malignant

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cells in CCA. The three-gene signature associated with monocytes and ferroptosis can be used to predict the outcome of CCA, allowing patients with CCA to get individualized treatment.

However, this study has limitations, as the authors comprised scRNA-seq data and TCGA-CCA contains bulk RNA-seq and the study of the role of ferroptosis in CCA and its association with the tumor microenvironment was limited to these datasets. Even though the authors confirmed their findings using validation sets based on public datasets, validation in prospective studies will further support the validity of these results; additional investigation of the molecular mechanisms behind these observations is needed.

AUTHOR CONTRIBUTIONS
P.R.C. has conceived and written this commentary.

DECLARATION OF INTERESTS
The author has no conflict of interest to declare.

REFERENCES

1. Yao, W., Liu, X., He, Y., Tian, M., Lu, S., Wang, Q., Zheng, Y., Lv, Z., Hao, C., Xue, D., and Meng, X. (2022). ScRNA-seq and bulk RNA-seq reveal the characteristics of ferroptosis and establish a risk signature in cholangiocarcinoma. Mol. Ther. Oncolytics 27, 48–60.

2. Rizvi, S., and Gores, G.J. (2013). Pathogenesis, diagnosis, and management of cholangiocarcinoma. Gastroenterology 145, 1215–1229.

3. Chua, D., Low, A., Koh, Y., Goh, B., Cheow, P.C., Kam, J.H., Teo, J.Y., Tan, E.K., Chung, A., Ooi, L.L., et al. (2018). A retrospective review of correlative radiological assessment and surgical exploration for hilar cholangiocarcinoma. Ann. Hepatobiliary. Pancreat. Surg. 22, 216–222.

4. Li, J., Cao, F., Yin, H.L., Huang, Z.J., Lin, Z.T., Mao, N., Sun, B., and Wang, G. (2020). Ferroptosis: past, present and future. Cell Death Dis. 11, 88.

5. Zhang, M., Yang, H., Wan, L., Wang, Z., Wang, H., Ge, C., Liu, Y., Hao, Y., Zhang, D., Shi, G., et al. (2020). Single-cell transcriptomic architecture and intercellular crosstalk of human intrahepatic cholangiocarcinoma. J. Hepatol. 73, 1118–1130.

6. Gao, M., Monian, P., Pan, Q., Zhang, W., Xiang, J., and Jiang, X. (2016). Ferroptosis is an autophagic cell death process. Cell Res. 26, 1021–1032.