News Release

Fatal COVID-19 pulmonary disease involves ferroptosis

Key Points

• In COVID-19, severe pulmonary lesions can lead to mortality.
• We analyzed severe pulmonary lesions in COVID-19 using post-mortem autopsy samples from patients in the United States and an experimental animal model with hamsters.
• In severe pulmonary lesions of COVID-19, involvement of ferroptosis, an iron-dependent form of regulated cell death, has been demonstrated, suggesting its potential as a new drug target.

Summary

SARS-CoV-2 infection leads to severe lung damage, with ferroptosis, an iron-dependent cell death, playing a significant role. Hyperferritinemia and disrupted iron homeostasis in COVID-19 patients suggest ferroptosis involvement. Autopsy analysis revealed increased ferroptosis markers, lipid dysregulation, and ferritin accumulation in severe cases. In a hamster model, ferroptosis markers correlated with lung injury severity. These findings highlight ferroptosis as a key factor in COVID-19 lung pathology, suggesting that inhibiting ferroptosis could help prevent lung damage during infection.

Research Background

Coronavirus disease-2019 (COVID-19), caused by SARS-CoV-2, leads to severe lung conditions like pneumonia and acute respiratory distress syndrome (ARDS), which are major contributors to high mortality rates. Acute lung injury (ALI), including diffuse alveolar damage (DAD), and non-acute lung injury (non-ALI), such as pulmonary vascular congestion and microthrombi, are common in COVID-19 patients. These lung pathologies are driven by both viral damage and host inflammatory responses, including cytokine storms and oxidative injury from immune cells. Despite treatments like intubation, mechanical ventilation, and a combination of antiviral and anti-inflammatory medications, effective therapies for COVID-19 lung disease remain limited.

Ferroptosis, an iron-dependent form of cell death driven by phospholipid peroxidation, plays a significant role in various diseases. In COVID-19, several pro-ferroptotic changes have been observed. SARS-CoV-2 infection decreases

GPX4 expression, reducing the cell's ability to repair lipid peroxidation, while the virus also inhibits the NRF2 antioxidant response, increasing susceptibility to oxidative damage. Additionally, elevated serum ferritin in critically ill COVID-19 patients supplies iron that drives ferroptosis, disrupting iron metabolism in the lungs.

This study identifies ferroptosis as a major mechanism underlying COVID-19 lung pathology. By examining human COVID-19 lung tissue, we found distinct molecular markers of ferroptosis, particularly in severe ALI and non-ALI cases. Lipidomic analysis of lung autopsies revealed changes in lipids associated with inflammation, metabolism, and ferroptosis. Further investigation using a Syrian hamster model of COVID-19 showed a strong correlation between ferroptosis and lung pathology.

Research Results

The study explores the role of ferroptosis, a form of iron-dependent cell death, in the lung pathologies of COVID-19 patients. We analyzed lung tissue from severe COVID-19 cases, distinguishing between acute lung injury (ALI) and non-ALI types, as well as from non-affected and non-COVID-19 ALI controls. Microscopic examination identified hallmark features of ALI and non-ALI in COVID-19 lungs. The study aimed to determine the cell death mechanisms involved, focusing on ferroptosis, necroptosis, apoptosis, and pyroptosis.

Using immunofluorescence and immunohistochemistry, the study found that ferroptosis markers, transferrin receptor 1 (TfR1), and malondialdehyde adduct (MDA), were elevated in COVID-19 ALI and non-ALI lungs compared to control samples. In contrast, markers for necroptosis, apoptosis, and pyroptosis did not show increased levels, suggesting that ferroptosis is the predominant cell death mechanism in fatal COVID-19 lung disease. Validation using positive and negative controls further supported the specificity of these markers for ferroptosis. The study also examined the role of tumor suppressor p53 and E3 ligase MDM2, which are known to promote ferroptosis. However, these proteins were not elevated in COVID-19 lungs, indicating they do not drive the observed ferroptosis.

We then explored the connection between iron dysregulation and ferroptosis, noting that COVID-19 patients exhibited elevated serum ferritin, a marker of iron overload. In severe COVID-19 cases, both TfR1 and ferritin light chain (FTL) were significantly elevated, indicating iron dysregulation's role in promoting

ferroptosis. Analysis of gene expression in post-mortem COVID-19 lungs revealed that iron regulatory genes, especially FTL and TfR1, were highly expressed in macrophages, fibroblasts, and epithelial cells. In vitro experiments with lung epithelial cells treated with ferric ammonium citrate (FAC) showed that iron overload induces ferroptosis and lipid peroxidation, consistent with findings in human lung tissue.

Lipidomics analysis of COVID-19 lung autopsies further confirmed the presence of ferroptosis. The study identified significant alterations in 130 lipid species, with a depletion of phospholipids containing polyunsaturated fatty acids (PL-PUFAs) and an accumulation of lysophospholipids, both hallmarks of ferroptosis. Additionally, COVID-19 lungs showed depletion of surfactant phospholipids and an increase in free fatty acids and storage lipids, indicating disrupted lung function and cellular lipotoxicity.

Finally, the study used a Syrian hamster model of COVID-19 to assess ferroptosis during disease progression. The hamsters developed lung injury that correlated with increased ferroptosis markers such as TfR1 and 4-hydroxynonenal (HNE). Inhibition of ferroptosis with liproxstatin-1 reduced lung injury, suggesting that targeting ferroptosis could be a potential therapeutic approach.

Overall, the study provides strong evidence that ferroptosis is a key contributor to COVID-19 lung pathology, driven by iron overload and lipid peroxidation. These findings suggest that ferroptosis inhibition could be a valuable strategy in mitigating lung injury in severe COVID-19 cases.

Research Summary and Future Perspective

This study investigates the molecular mechanisms behind COVID-19-related lung damage, focusing on ferroptosis as the primary form of cell death contributing to this damage. Ferroptosis is characterized by iron dysregulation, lipid peroxidation, and depletion of polyunsaturated fatty acids (PUFAs). The study examined lung tissues from deceased COVID-19 patients and a hamster model of the disease, finding significant markers of ferroptosis, particularly in areas of severe lung damage. This suggests that ferroptosis plays a crucial role in the progression of lung disease in COVID-19.

In COVID-19 patients, lung damage includes diffuse alveolar damage (DAD), congestion, and blood clotting, with blood accumulation in lung tissues

contributing to disease severity. High levels of serum ferritin and ferritin light chain in the lungs of severe cases indicate iron overload, which exacerbates tissue damage through ferroptosis. The study found that primary lung epithelial cells, when exposed to iron overload, became more susceptible to ferroptosis, particularly when combined with other stressors, highlighting the role of iron in promoting this destructive process during SARS-CoV-2 infection.

The research also used advanced bioinformatics, including single-nucleus transcriptomic profiling, to identify key genes and pathways linked to COVID-19-induced lung injury. These included oxidative stress responses and signaling pathways related to ferroptosis. Additionally, lipidomics analysis revealed significant changes in lung lipid composition, particularly the depletion of phospholipids and surfactant components essential for lung function. These changes reflect increased oxidative stress and lipid peroxidation, further contributing to lung damage.

The study also examined the potential for ferroptosis inhibitors to reduce lung injury. While these inhibitors partially mitigated lung damage in a hamster model, their effectiveness was limited by poor lung accumulation. This suggests that direct pulmonary delivery methods, such as inhalation, might be more effective in targeting lung lesions and preventing ferroptosis.

In conclusion, ferroptosis plays a significant role in COVID-19 lung pathology, driven by iron overload and oxidative stress. Targeting ferroptosis may offer a therapeutic approach to preserve lung function in COVID-19 patients, and ferroptosis markers could potentially be used to assess disease severity.

Publication

Fatal COVID-19 pulmonary disease involves ferroptosis.
Qiu B, Zandkarimi F, Saqi A, Castagna C, Tan H, Sekulic M, Miorin L, Hibshoosh H, **Toyokuni S**, Uchida K, Stockwell BR. Nat Commun. 2024 May 20;15(1):3816. doi: 10.1038/s41467-024-48055-0. PMID: 38769293

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