Clinical significance of ferroptosis as iron-dependent regulated cell death in the general structure of the disease

Abstract. In this article, we have analysed the studies that determined the iron-dependent regulated type of cell death, ferroptosis, described the fundamental morphological and biochemical differences between various types of regulated cell death, highlighted modern scientific achievements in understanding the features of the above-mentioned process, described the clinical significance of ferroptosis in the general structure of morbidity and identified relevant issues for further research. Conclusions. Numerous studies allowed identifying ferroptosis as a form of regulated cell death, initiated by oxidative disturbances of the intracellular microenvironment, which is under the constitutive control of glutathione peroxidase 4 and can be inhibited by iron chelators and lipophilic antioxidants. Ferroptosis can occur in two main ways: external (transport) and internal (enzymatic). The external pathway is based on non-enzymatic reactions, such as the iron-dependent Fenton reaction. The internal pathway is mediated by enzyme systems, including glutathione peroxidase 4 and lipoxygenase. Conducting clinical research will improve not only the understanding of the role of ferroptosis in the pathogenesis of the course of diseases, but also reveal possible preventive strategies for the development of pathological processes.

Keywords: ferroptosis; cell death; review

An excess of free reactive iron can cause various types of cell death, including the recently recognized type — ferroptosis. The field of ferroptosis research has grown exponentially over the past few years since the term was coined in 2012.

The definition of ferroptosis as a separate form of cell death was preceded by studies from 1955 [1] the stages of which can be conventionally distinguished as:


Although the main molecular effector of the process remains poorly understood, it is known that ferroptosis is induced by the activation of iron-dependent lipid peroxidation [2]. Significant progress has been made in analyzing the mechanisms that lead to lipid peroxidation and how antioxidant systems or stress proteins regulate ferroptosis. Ferroptosis is a special variant of cell death in view of its important role in the initiation and development of diseases, including inflammatory diseases, and the determination of its inhibitors allows evaluating the preventive effect on experimental models of certain pathological processes, such as cancer. However, some studies suggest that ferroptosis may be a physiological process that occurs widely in the body of mammals, rather than a pathological or organ-specific one [3].

However, the scientific community today defines ferroptosis as an iron-dependent programmed cell death pathway, mainly due to redox imbalance, which has categorically different biological and morphological characteristics compared to other cell death patterns [4].

Given the fact that apoptosis was the first discovered and studied mechanism of regulated cell death, necrosis and py-

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For corresponding: Hennadii Lezhenko, MD, Professor, Head of the Department of Hospital Pediatrics, Zaporizhzhia State Medical University, Mayakovsky avenue, 26, Zaporizhzhia, 69035, Ukraine; e-mail: genalezh@gmail.com

Full list of authors information is available at the end of the article.
Ferroptosis were recently distinguished alongside it [5]. However, ferroptosis categorically differs from apoptosis, necrosis and pyroptosis in terms of morphological and physiological characteristics [5–14]. Currently, ferroptosis is classified by a number of mechanisms (necroptosis, pyroptosis, NETosis, parthanatos), defined as “regulated necrosis” [15, 16]. Unlike apoptosis, which is characterized by cytoplasm shrinkage, nuclear division, chromatin condensation, chromosomal DNA division [8, 14] and the release of mitochondrial cytochrome C [15], and necrosis, which is characterized by cytoplasmic granulation, swelling of organelles and cells, loss of cell membrane integrity and, ultimately, leakage of cellular contents [16], during ferroptosis, cell nuclei remain intact, chromatin does not aggregate [17, 18], the plasma membrane does not rupture, and contracting mitochondria exhibit a greater inner membrane density while the outer membrane ruptures. Ultrastructural analysis also showed that the mitochondria of ferroptotic cells lose structural integrity and have a different morphological structure (smaller size) [19], whereas mitochondria in apoptotic cells are usually “bloated” [20]. Unlike apoptosis, ferroptosis exhibits immunogenicity [21], as affected cells release damage-associated molecular patterns and alarms, which enhance cell death and promote a number of reactions associated with inflammation [15, 22–24].

It was determined that the precursor to the understanding of the process of ferroptosis was oxytosis, which had several characteristics in common with the latter, such as: the role of lipoxigenase, the production of reactive oxygen species, and gene expression [25]. However, the discovery of the compound erastin, which was able to initiate ferroptotic cell death, played a significant role in identifying the mechanism of ferroptosis [19]. Stockwell et al. (2017) research [26] allowed identifying ferroptosis as a form of regulated cell death, initiated by oxidative disturbances of the intracellular microenvironment, which is under the constitutive control of glutathione peroxidase 4 and can be inhibited by iron chelators and lipophilic antioxidants [14]. Glutathione peroxidase 4 is one of the central regulators of ferroptosis [27]. Glutathione acts as a cofactor of glutathione peroxidase 4 and maintains its level by exchanging glutamate and cystine through the \( \chi^- \)-antiporter system [26, 27]. Studies of ferroptosis have shown the crucial role of mitochondria in its occurrence through lipid metabolism, energy metabolism, iron metabolism and other regulatory processes in mitochondria [28]. The decreased reduction of lipid peroxides caused by the inhibition of glutathione peroxidase 4, and increased formation of lipid peroxides from arachidonoyl are the two main pathways that lead to ferroptosis [3]. Further studies showed that ferroptosis is mediated by mitochondrial voltage-dependent anion channels (VDACs), and erastin-induced opening of VDAC2/3 leads to mitochondrial iron uptake, reactive oxygen species formation, increased mitochondrial potential, and oxidative stress-induced ferroptosis [6, 29]. Since this discovery, the complex interplay between iron, cysteine and lipid metabolism has emerged as an important regulator of ferroptosis [30], and a number of its regulators have been identified. Other organelles besides mitochondria are also involved in ferroptosis. Oxidative stress associated with the endoplasmic reticulum, Golgi stress-related lipid peroxidation, and lysosomal dysfunction contribute to the induction of ferroptosis [31].

The most important biochemical features of ferroptosis are increased levels of lipid hydroperoxides and concentrations of ferrous ions (Fe\(^{2+}\)), as ferroptotic cells produce an excessive amount of reactive oxygen species, which initiates lipid peroxidation through the Fenton reaction, and an enzymatic mechanism with the participation of lipooxygenase [21]. The Fenton reaction partially explains the dependency of ferroptosis on iron, as redox pools of iron can directly catalyze the spread of lipid peroxidation with the synthesis of pathological compounds that will induce the release of cellular iron [1].

The genetic control system of ferroptosis is excellent compared to other types of cell death. Six genes encoding putative mitochondrial proteins, ribosomal protein L8 (RPL8), iron-responsive element-binding protein 2 (IREB2), ATP synthase, citrate synthase (CS) proteins, tetratricopeptide repeat domain 35 (TTCS35) and acyl-CoA synthetase family member 2 (ACSF2) were isolated. In addition, TFRC, ISCU, FTTH1, and FTL are key ferroptosis genes that control erastin sensitivity, iron uptake, metabolism, and storage by influencing Fe\(^{2+}\) levels [24]. Dixon et al. (2012) identified the specific role of RPL8, IREB2, ACSF3, TTC35, CS, and ACSF2 in erastin-induced ferroptosis [19].

Translational and transcriptional regulation of iron homeostasis provide an integrated network for determining ferroptosis sensitivity [32]. The iron-dependent mechanism of ferroptosis creates conditions for peroxidation of phospholipid membranes rich in polyunsaturated fatty acids, which leads to cell death. Understanding the mechanism of lipid peroxidation emphasizes the important role of iron and reactive oxygen species in ferroptosis. After all, iron is a redox metal that participates in the formation of free radicals and the spread of lipid peroxidation, and therefore, an increase in its level can increase the vulnerability to ferroptosis [33]. Inhibition of SLC7A11 and glutathione peroxidase 4 leads to accumulation of iron-dependent lipid peroxidation, thus causing the death of ferroptotic cells. The specific iron-dependent mechanisms of ferroptosis remain poorly understood, but currently we can determine the unconditional role of the following components of iron metabolism:

- iron chelators block the death of ferroptotic cells in \textit{vitro} and \textit{in vivo} [19];
- an increase in cellular labile iron is usually observed during the induction of ferroptosis [34];
- exogenic iron supplementation increases the sensitivity of cells to inducers of ferroptosis (for example, erastin) [19];
- an excess of heme and non-heme iron can directly induce ferroptosis [35];
- several heme and non-heme iron-containing enzymes, such as ALOX, NOX and CYP, are responsible for the process of lipid peroxidation [18, 19, 36–38];
- iron-mediated production of reactive oxygen species via the Fenton reaction contributes to lipid peroxidation in ferroptosis [24].

The transporter protein transferrin is necessary for the induction of ferroptotic cell death. For example, cell death
caused by amino acid deficiency takes the form of ferroptosis instead of apoptosis or necroptosis. This amino acid starvation-induced ferroptosis is likely a consequence of cysteine starvation and subsequent cellular depletion of GSH. A substance without macromolecules (for example, transferrin) is unable to mimic ferroptosis-inducing activity, while the addition of recombinant iron-saturated holotransferrin induces cell death under the same conditions [39]. These findings indicate that transferrin is a key positive regulator of the ferroptosis process [32].

Importantly, all aspects of iron metabolism, including iron uptake, storage, export, and utilization, have important regulatory effects on ferroptosis [2].

A common feature of ferroptosis is the iron-dependent accumulation of lipid reactive oxygen species and subsequent depletion of phospholipids and polyunsaturated fatty acids [40]. Chains of polyunsaturated fatty acids of membrane lipids are more susceptible to both enzymatic and non-enzymatic oxidation, which leads to their fragmentation [41]. In turn, reactive oxygen species include singlet oxygen molecules and three types of free radicals: hydroxyl radicals, superoxide anions and hydrogen peroxide. Reactive oxygen species regulate several cellular functions at optimal concentrations and homeostatic balance. However, at critically high concentrations, reactive oxygen species can cause DNA damage, protein denaturation, and induce lipid peroxidation [42]. In this process, catalyzed by iron and oxygen, membrane destruction and cell death occur [21].

Today, ferroptosis is defined as a pathogenetic factor of damage to the tissues of the brain, heart, liver, and renal tubules, blood flow stoppage, sleep apnea, which developed against the background of tissue ischemia/reperfusion [42]. Ferroptosis disrupts the normal immune response by killing T-lymphocytes, which has been proven in mice. The ferroptotic process is a companion of neurodegenerative diseases, which is not surprising, since nerve cells are distinguished by the maximum content of polyunsaturated fatty acids, and some pathologies of the nervous system, including Alzheimer’s, Parkinson’s and Huntington’s diseases, are due to the inability to restore oxidized lipids. Some clinical studies demonstrated an increased iron level in the brain of children with severe ischemic-anoxic stroke [43]. In addition, it was found that elevated level of iron against the background of ischemia/perfusion is a mediator of tissue damage. In support of this scientific claim, reduction of brain damage in response to iron chelation has been demonstrated in several animal models [44].

Interestingly, recent studies have shown that ferroptosis is also involved in the development of inflammatory bacterial diseases. Anthonymuthu T. et al. (2021) found that Pseudomonas aeruginosa can cause ferroptosis in human bronchial epithelial cells by generating lipoygenase [45]. Observations are described on the role of Mycobacterium tuberculosis in ferroptotic cell damage by inhibiting glutathione peroxidase 4 [46]. Unlike immunologically silent cells that undergo apoptosis, ferroptotic targets are inherently more immunogenic because they release inflammatory cytokines and damage-associated molecular patterns, skewing the environment to a pro-inflammatory state [22].

Our study made it possible to analyze the likely role of ferroptosis in the development of anemia of inflammation in young children with acute inflammatory bacterial diseases of the respiratory organs [47, 48].

However, we believe that it is wrong to perceive ferroptosis exclusively as a pathological process. It is appropriate to recall that ferroptosis was determined directly during research aimed at the treatment of oncological diseases [19]. Modern data on its ability to inhibit the growth of tumors allow expanding the understanding of the role of ferroptosis in the body.

Therefore, the study of ferroptosis allows establishing a new scientific platform, which can be aimed at studying the clinical significance of ferroptosis in the development of a number of pathological processes, determining new preventive and treatment strategies for diseases, and answering the following questions:

— The effect of ferroptosis on the inflammatory reaction is not absolute; under what circumstances will the induction of ferroptosis have a pro-inflammatory or anti-inflammatory effect?
— Is it possible to determine the ways to regulate ferroptosis, which will allow improving treatment tactics?
— Since ferroptosis is closely related to inflammation and oncological process, does it play a role in inflammation-mediated carcinogenesis?

**Conclusions**

1. Numerous studies allowed identifying ferroptosis as a form of regulated cell death, initiated by oxidative disturbances of the intracellular microenvironment, which is under the constitutive control of glutathione peroxidase 4 and can be inhibited by iron chelators and lipophilic antioxidants.

2. Ferroptosis can occur in two main ways: external (transport) and internal (enzymatic). The external pathway is based on non-enzymatic reactions, such as the iron-dependent Fenton reaction. The internal pathway is mediated by enzyme systems, including glutathione peroxidase 4 and lipoygenase.

3. Conducting clinical research will improve not only the understanding of the role of ferroptosis in the pathogenesis of the course of diseases, but also reveal possible preventive strategies for the development of pathological processes.

**References**


Information about authors
Hennadii Lezhenko, MD, Professor, Head of the Department of Hospital Pediatrics, Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine; e-mail: genalezh@gmail.com; https://orcid.org/0000-0003-0851-4586
Aleksandr Abaturov, MD, PhD, Professor, Head of the Department of Pediatrics 1 and Medical Genetics, Dnipro State Medical University, Dnipro, Ukraine; https://orcid.org/0000-0001-6291-5386
Pogribna A.O., PhD, Assistant at the Department of Hospital Pediatrics, Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine; e-mail: a.pogrebnaia@gmail.com

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Леженко Г.О.1, Абатуров О.Є.2, Погрібна А.О.1
1Запорізький державний медичний університет, м. Запоріжжя, Україна
2Дніпровський державний медичний університет, м. Дніпро, Україна

Клінічне значення фероптозу як залізозалежної регулюваної загибелі клітин у загальній структурі захворювання

Резюме. У цій роботі ми проаналізували дослідження, у яких було визначено залізозалежний регулюваний тип клітинної загибелі — фероптоз, описали принципові морфологічні й біохімічні відмінності різних варіантів регулюваної загибелі клітин, висвітлили сучасні наукові досягнення щодо розуміння властивостей перебігу вищевказаних процесів, описали клінічне значення фероптозу в загальній структурі захворюваності та визначили актуальні питання подальших досліджень.

Висновки. Численні дослідження дозволили ідентифікувати фероптоз як форму регулюваної клітинної загибелі, ініціювану окиснювальними порушеннями внутрішньоклітинного мікроочінення, що знаходиться під конститутивним контролем глутатіонпероксидази-4 і може бути інгібована хелаторами заліза та ліпофільними антиоксидантами. Фероптоз може бути реалізований двома основними шляхами: зовнішнім (транспортним) та внутрішнім (ферментним). В основі зовнішнього шляху лежать неферментативні реакції, такі як залізозалежна реакція Фентона. Внутрішній шлях відбувається під посередництвом ферментних систем, що включають глутатіонпероксидазу та ліпоксигеназу. Проведення клінічних досліджень дозволить удосконалити не лише розуміння ролі фероптозу в патогенезі перебігу захворювань, але й виявити можливі предуспільні стратегії щодо розвитку патологічних процесів.

Ключові слова: фероптоз; клітинна загибель; огляд