

GPX4 Shields Organs from Sepsis-induced Damage by Regulating Programmed Cell Death

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Abstract: Sepsis, a condition characterized by multi-organ dysfunction resulting from a severe imbalance of inflammation following infection, is notorious for its high fatality rate, poor prognosis, and the intricate challenge of estimating its annual economic losses. Nevertheless, the intricate mechanisms underlying sepsis remain elusive. Programmed cell death, an umbrella term, embraces diverse modalities such as apoptosis, necroptosis, autophagy, ferroptosis, pyroptosis, and recently identified cuproptosis. Among them, GPX4 (glutathione peroxidase 4) emerges as a key intracellular regulator intricately involved in various physiological processes such as ferroptosis, cuproptosis, autophagy, apoptosis, necroptosis and pyroptosis crucial in maintaining REDOX balance in the body. GPX4 plays a vital role in safeguarding against sepsis-induced lung injury and other organ damage through the regulation of ferroptosis, pyroptosis and other programmed cell deaths. This treatment option can potentially rescue critically ill patients suffering from severe infections and enhance the survival rate of patients in the intensive care unit. The present study endeavors to decipher the molecular underpinnings of GPX4's role in sepsis and uncover potential therapeutic targets for the treatment of this devastating condition.

Keywords: GPX4; Sepsis; Organ Protection; Programmed Cell Death.

1. Introduction

Sepsis, recognized as a global health challenge by the World Health Organization (WHO), is defined by life-threatening organ dysfunction caused by an impaired response of the host to infection[1]. Sepsis is defined as the body's uncontrolled inflammatory response to an infection that leads to the failure of various organs and is life-threatening. Sepsis has a high incidence and fatality rate. Almost 14,000 individuals succumb to sepsis daily across the globe[2]. Sepsis occurs when the body's response to infection-induced inflammation becomes unbalanced, resulting in life-threatening multiple organ dysfunction(MODS)[3]. Although the study of toxicosis has made great progress in recent years, its pathophysiology is not fully understood[4]. The immune response to sepsis is complex and is commonly characterized as having two components. The initial element is the hyperinflammatory condition characterized by a systemic inflammatory reaction to an infection[5]. DAMPs interact with pattern recognition receptors on immune cells, triggering the release of inflammatory cytokines and chemokines, and the recruitment of immune cells to the inflamed area, ultimately causing heightened inflammation and organ dysfunction[6]. The second component is the immunosuppressed state, which is the vulnerable stage of secondary infection[7]. Regardless of immune and non-immune cells, various forms of cell death play a key role in the excessive inflammatory and immunosuppressive state during sepsis[8]. Enhanced and prolonged inflammation in sepsis results in both programmed and unprogrammed cell death, causing not only organ damage but also prolonged immunosuppression that increases vulnerability to infections[9]. Many studies have demonstrated that RCD (programmed cell death) occurs in immune cells and plays a crucial role in immune dysfunction during sepsis. RCD-induced changes in cellular permeability and release of

intracellular contents can lead to excessive inflammatory responses or immune impressions[10].

GPX (glutathione peroxidase) belongs to a protein superfamily whose catalytic site contains cysteine as a REDOX active residue to regulate REDOX reactions[11]. The human GPX consists of eight members, each with different structural, functional, and expressive characteristics[12]. Among them, GPX4, originally known as PHGPX (phospholipid hydrogen peroxide), was first purified from pig liver in 1982 by Ursine et al[13]. It utilizes reductive equivalent glutathione (GSH) to protect liposomes and biofilms from oxidation[14]. GPX4's structure and function establish it as a crucial intracellular regulator that plays a role in multiple physiological processes[15]. Recent studies have found an important role for GPX4 in sepsis, a key antioxidant enzyme that plays an important role in regulating programmed cell death such as ferroptosis, pyroptosis, and apoptosis. In addition, it can mediate developmental defects, tissue damage, and aseptic inflammation[16]. Over the past decade, GPX4 has garnered significant interest in fields such as oncology, cardiovascular research, and neuroscience. Revealing the mechanism of GPX4 activity regulation has emerged as a prominent research area.

2. GPX4 Regulates Programmed Cell Death

2.1. GPX4 and Ferroptosis

Ferroptosis stands out as a distinct form of programmed cell death, distinguishing itself from apoptosis, pyroptosis, necroptosis and other mechanisms of cellular demise[17]. Ferroptosis is dependent on the presence of iron and lipid peroxidation, leading to the build-up of iron within cells and the rise of lipid peroxidation byproducts[18]. This process ultimately culminates in cell damage and death. It is propelled by iron-dependent lipid peroxidation and influenced by a

myriad of cellular metabolic pathways, encompassing REDOX balance, iron metabolism, mitochondrial function, and the processing of amino acids, lipids and sugars, alongside various signaling pathways linked to disease[19]. It is distinguished morphologically by mitochondrial contraction and an increase in mitochondrial membrane[16]. In typical circumstances, the iron ion-based oxidation system, Fenton reactions, and reactive oxygen species (ROS) work in opposition to the antioxidant system comprised of cystine/glutamate reverse transporters (system Xu –/GSH/GPX4 axis) to regulate cellular and bodily balance. Increased iron buildup and oxidation of lipids are key factors that initiate iron-induced cell death[20]. Chelating excess iron and activating both the GPX4-dependent and independent antioxidant pathways are effective strategies to prevent iron-induced cell death[15]. Therefore, at the core of iron-induced cell death lies the disruption of REDOX balance[21]. GPX4 plays a crucial role in this process, regulating lipid peroxidation, converting lipid peroxides to lipid alcohols, and inhibiting REDOX imbalances induced by ferroptosis.

2.2. GPX4 and Pyroptosis

Pyroptosis is an inflammatory type of programmed cell death where cells undergo continued expansion until their membrane bursts, leading to the release of cellular contents that stimulate a robust inflammatory and immune reaction[22]. The main death signaling pathway revolves around two branches the classical pathway activated by caspase-1 and the non-classical pathway activated by caspase-4/5/11[23]. Both pathways utilize GSDMD as downstream effectors. In recent studies, the classical pathway has been shown to not only detect pathogen-associated molecular patterns(PAMPs)and injury-associated molecular patterns(DAMPs),but also recognize cytoplasmic disorders, collectively referred to as molecular events that disrupt homeostasis(HAMPs)[24]. PAMPs and DAMPs are recognized within the cell by cytoplasmic pattern recognition receptors, which activate inflammasome complexes that assemble to perform pyroptosis[25]. Upon binding to a PAMP or DAMP, the PRRs engage and trigger the activation of an apoptosis-related ASC protein, which subsequently forms oligomers and utilizes its caspase activation and recruitment domain(CARD)to interact with the CARD of pro-caspase1[26].The complex that binds PRRs, ASC and procaspase-1 is called the inflammasome[27]. The CARD-CARD interaction triggers the activation of pro-caspase-1, thereby cutting GSDMD and pro-IL-1 β /IL-18 into their active forms, GSDMD is cut into two domains, GSDMD-N and GSDMD-C, and GSDMD-N selectively interacts with membrane lipids to form transmembrane pores[28]. The release of danger signals such as DAMPs (e.g. IL-1 β , IL-18 and other cytokines) through the release of cell contents across membrane pores[28]. Non-conventional techniques were employed to identify the intracellular lipopolysaccharides (LPS)of Gram-negative bacteria[29]. LPS directly activates Pro-caspase-11 (human Pro-caspase4/5) by binding to CARD and is activated into caspase11 through oligomerization and proteolysis[30]. Like Caspase-1, activated caspase11 cuts the junction region of GSDMD[31]. GSDMD-N and GSDMD-C are separated to induce cell death. The non-classical pathway interconnects with the NLRP3 inflammasome via pyrin domain 3, triggering caspase-1 activation, cleavage of pro-IL-1 β /IL-18, and the induction of inflammation[32].

The antioxidant enzyme GPX4 inhibits lipid peroxidation by breaking down hydrogen peroxide and shields mice from septic death[16]. The expression of GPX4 in myeloid cells (especially macrophages) limits caspase-1/caspase-11 mediated inflammatory body activation and GSDMD-dependent pyroptosis[33]. In different experimental models, GPX4 has been demonstrated to block the activation of the inflammatory NF- κ B pathway and the generation of pro-inflammatory mediators like IL-1B and PTGS2/COX-2, hence averting cytokine storms and ultimately saving lives from sepsis during bacterial infections[15].

2.3. GPX4 and Necroptosis

Necroptosis is a form of programmed cell death initiated by a complex composed of receptor-interacting protein kinase 1(RIPK1) and receptor-interacting protein kinase 3 (RIPK3), characterized by extensive leakage of cytoplasmic contents and swelling of organelles[34]. Necroptosis has a strong association with various receptors. Death receptors like TNFR1,CD95,and TRAIL-R are implicated in this process[35]. In addition, Toll-like receptors such as TLR3 or TLR4 also play a role. Moreover, Z-DNA binding protein 1 (ZBP1/DAI/DLM-1) in the cytoplasm is also related to necroptosis[36, 37]. When tumor necrosis factor alpha(TNF- α)interacts with tumor necrosis factor receptor 1(TNFR1),TNFR1 begins to recruit downstream protein molecules. These molecules include TNFR1-associated death domain protein (TRADD), apoptosis protein cellular inhibitor(cIAPs), TNFR-associated factors 2/5, and linear ubiquitin chain assembly complex proteins, forming complex. In this complex, RIPK1 undergoes polyubiquitination[34]. Then it activates the nuclear factor- κ B(NF- κ B)and mitogen-activated protein kinase(MAPK)signaling pathways, thereby inhibiting the activation of caspase-8 and promoting cell survival[38]. However, when caspase -8 and/or the apoptotic protein cIAP is removed, these receptors activate RIPK1/3 and directly induce necroptosis[38, 39]. In the process of RIPK1-dependent necroptosis, RIPK1 undergoes autophosphorylation upon activation and interacts with RIPK3 through RIP homology interaction motif (RHIM)-RHIM interaction[40]. The complex formed by RIPK1 and RIPK3, commonly referred to as the necrosome, directly phosphorylates MLKL (a pseudokinase known as mixed lineage kinase-like). Phosphorylated MLKL assembles into oligomers and migrates to the cell membrane. Here, it creates pores that result in the loss of cell integrity and the release of intracellular substances[41, 42]. These substances, known as damage-associated molecular patterns(DAMPs)such as high mobility group protein B1(HMGB1),mitochondrial DNA, uric acid, histamine and ATP, are released into the extracellular space, leading to intense inflammation, the recruitment of white blood cells, and a prolonged immune response[43]. It also involves stimulating the innate immune system via pattern recognition receptors. Although necroptotic cell death plays a key role in host defense by hindering the growth of intracellular pathogens, it can also lead to uncontrolled inflammation and tissue damage.

Glutathione peroxidase 4(GPX4) plays a role in preventing RIP3-mediated necroptosis. While the mechanisms of cell death differ among various cell types, GPX4 plays a vital role in maintaining the balance of hematopoietic cells by regulating cellular redox levels and preventing cell death[44]. However, it is currently unclear how different cell death pathways cross or can be substituted due to genetic or

environmental changes[45]. Many studies have shown that there are complex interactions between death pathways when regulatory components such as FADD, caspase 8, RIP1, and RIP3 undergo genetic alterations[46-48]. The role of necrosome, TNF- α , FAS ligand, and DNA damage-dependent PARP1 activation in inducing GPX4-deficient cell death is insignificant[44]. GPX4 induces apoptosis by upregulating EGR1 in TNBC cells, thereby blocking the path of cells towards necroptosis[49]. A crucial requirement appears to be the functional inactivation of caspase 8 in cells lacking GPX4, potentially aided by glutathione[44]. Glutathione is also thought to regulate caspase -1 and caspase -3 as it is a byproduct of oxidation[50, 51]. Therefore, our results may be related to the activation of the TNF-A-dependent apoptotic pathway. This is because reactive oxygen species (ROS) generated after RIP1/RIP3 activation may disrupt the function of caspase 8 and promote a positive feedback loop[52].

2.4. GPX4 and Apoptosis

Initiation of apoptosis encompasses two primary pathways[53]. The initial pathway entails the communication of death signals via cell surface receptors. This route is known as the exogenous pathway and is controlled by caspase-8. Caspase-8 can be activated by various death ligands from the TNF superfamily, such as TNF-A, Fas and Dr3[54-57]. The second apoptosis pathway (endogenous pathway) is activated by mitochondrial damage and is mediated by caspase-9. The pathway mediated by mitochondrial caspase-9 can be triggered by various stimuli, such as reactive oxygen species, radiation and chemotherapy drugs, which are believed to interfere with the mitochondrial membrane potential[58, 59]. Both caspase-8 and caspase-9 ultimately trigger the activation of caspase-3, which participates in the final common pathway of cell death mechanisms[60-62]. It is crucial to comprehend the precise mechanism through which sepsis triggers apoptosis, as this knowledge can offer valuable insights into the factors that initiate cell death and facilitate the development of more focused treatment approaches[63].

The enzyme glutathione peroxidase 4(GPX4), which is a selenium-containing protein, harbors a unique selenocysteine residue at its catalytic site[33]. It catalyzes the reduction of lipid peroxides by consuming reduced glutathione and functions as an important molecule in the defense system against iron death[33]. GPX4 has been demonstrated to prevent apoptosis in certain malignancies, including glioma and breast cancer[51]. An earlier report by Zhao et al. Stated that reducing GPX4 expression leads to the initiation of apoptosis in glioma cells[64]. Ding et al reported that DMOCPTL, a derivative of the natural product feverolide, could induce iron death in triple-negative breast cancer (TNBC) cells through GPX4 ubiquitination[49]. Photodynamic therapy (PDT) functions by stimulating the P53/SLC7A11/GPX4 signaling pathway. This stimulates apoptosis and ferroptosis in cholangiocarcinoma cells while hindering the growth of the cancer.[65]. Yet, the involvement of GPX4 in the progression of apoptosis is not well understood. Research on the mechanism of sepsis mediated by apoptosis is still quite limited at present. Focusing on current research can help solve clinically immune-related tumor and infection problems.

2.5. GPX4 and Autophagy

Autophagy is a highly evolutionarily conserved cell death

mode and plays an important role in regulating the balance of inflammatory responses[66]. Autophagy primarily leverages the standard function of lysosomes to break down and recycle intracellular macromolecules and damaged cells. It mainly acts as a self-protection mechanism in physiological activities and can remove misfolded proteins and aged organelles in normal cells, maintaining the homeostasis of cells themselves[67]. As a mainly self-protection mechanism in physiological activities, autophagy is mainly divided into the following three forms according to the way to lysosomes: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA)[68]. Recent studies suggest that ferroptosis is a mode of cell death that depends on autophagy. In some cancer cells, TMEM164 influences GPX4 degradation and ATG12-ATG5-ATG16L1 complex formation induced by ferroptosis activators[17, 69]. Under starvation induction, ATG9A mediates the assembly of the ATG12 – ATG5-ATG16L1 complex[70]. HPCAL1 is a specific autophagy receptor related to ferroptosis in cancer cells and participates in CDH2 degradation. TAX1BP1 specifically mediates autophagic degradation of GPX4 in copper-induced ferroptosis[71]. Different from GPX4 degradation in response to erastin mediated by SQSTM1, exogenous copper can cause GPX4 to be recognized by TAX1BP1 by binding to cysteine in GPX4. The interaction between GPX4 and HSPA8/HSC70 or LAMP2A can promote CMA-mediated GPX4 degradation in HT1080 cancer cells[72]. CKB can mediate GPX4 phosphorylation and inhibit CMA-mediated GPX4 degradation[73]. Overexpression of GPX4 can reduce cardiolipin peroxidation and prevent the formation of PLOOHs, thereby inhibiting autophagosome formation[74]. L-carnitine has the potential to boost the mRNA levels of GPX4 and promote autophagy[75]. In yeast, 12-hydroxyicosatetraenoic acid is a substrate for Atg8 lipidation required for activating autophagy and may be related to GPX4 in mammals[76]. In conclusion, further clarifying the relationship between GPX4, autophagy, and lipid metabolism is very important for understanding cell fate.

2.6. GPX4 and Cuproptosis

A new study shows that excessive copper in cells can induce a unique form of cell death, namely cuproptosis[77]. The mechanism of cuproptosis is completely different from apoptosis and other forms of controlled cell death. Excessive copper specifically targets and damages a subset of metabolic lipoyl enzymes within the tricarboxylic acid (TCA) cycle. The key indicators of cuproptosis are the abnormal buildup of dihydrolipoamide S-acetyltransferase (DLAT) protein and the resulting depletion of iron-sulfur (Fe-S) cluster proteins, which cause protein toxic stress and ultimately lead to cell death[78, 79]. Cuproptosis exists in all organisms, and its main mode of action may be to exert physiological functions through its oxidized state Cu (II) and reduced state Cu(I)[80, 81]. Due to its inherent redox properties, the regulation of intracellular copper homeostasis can be strictly controlled and may manifest differently in different diseases[82, 83]. Just as the absence of the copper ion transmembrane transporter ATP7B is considered related to the pathogenesis of Wilson's disease[83], it can lead to abnormal accumulation of free copper, ultimately destroying copper homeostasis and causing cell death. Cuproptosis is associated with a range of diseases, including Wilson's disease, neurodegenerative disorders, cancer and cardiovascular diseases. Copper levels are

strongly associated with the occurrence and mortality rates of cardiovascular diseases. Studies have found that ferredoxin 1 (FDX1) plays a key role in copper-induced death. FDX1 is an enzyme that converts Cu^{2+} to the more harmful form Cu^{+} . They also found that FDX1 is involved in protein lipoylation highly conserved post-translational modification of mitochondrial enzymes and is crucial for mitochondrial metabolism[84]. In addition, copper directly binds to lipoylated mitochondrial enzymes (especially dihydrolipoamide S-acetyltransferase) and interferes with iron-sulfur (Fe-S) cluster proteins in an FDX1-dependent manner. This results in the accumulation of lipoylated proteins, subsequently leading to the degradation of Fe-S cluster proteins[84]. This subsequently initiates toxic stress in mitochondrial proteins, ultimately resulting in cell death. Mitochondrial energy metabolism plays a regulatory role in cuproptosis. DSF/Cu strongly accelerates the production of a large amount of ROS, destroys mitochondrial morphology, accelerates the TCA cycle, aggregates lipoylated proteins, and induces the loss of proteins containing Fe-S clusters. Both copper plasmid protein inhibitors (TTM) and GSH can reduce cell death caused by DSF/Cu incubation[85]. Inhibition of the xCT-GSH-GPX4 pathway can sensitize DSF/Cu-induced MDS cell death. The interaction between ferroptosis and cuproptosis is affected by the important role of GSH. GSH can sequester excess copper ions through its sulfhydryl (-SH) group and completely reverse DSF/Cu-induced cell death[85]. Ferroptosis is caused by the accumulation of liposome oxides (LPO) induced by the enrichment of intracellular Fe^{2+} and the activation of the GPX4 pathway induced by the depletion of $\text{Fe}^{3+}/\text{Cu}^{2+}$ of GSH[86, 87]. In turn, enriched Cu^{2+} binds to and oligomerizes dihydrolipoamide S-acetyltransferase (DLAT) in mitochondria, triggering the destruction of the tricarboxylic acid cycle (TCA) and activating the cellular cuproptosis pathway[88, 89]. Therefore, in current studies, the regulation of cuproptosis by GPX4 mainly plays a role through its coordinated effect with ferroptosis, and more related mechanisms can be used as the direction for follow-up research.

3. GPX4 and Sepsis

Sepsis is a critical illness that is primarily triggered by the host's uncontrolled reaction to infection. characterized by organ dysfunction[90]. Its characteristics are severe systemic inflammation and multi-organ dysfunction (such as blood vessels, lungs, liver, kidneys, heart, and brain) caused by the uncontrolled infection of the host[1]. Sepsis is a serious and acute condition with a high mortality rate that can affect multiple organs throughout the body. GPX4 plays an important role in inhibiting lipid peroxidation in subcellular structures such as the plasma membrane, mitochondria, and nucleus, and is of great significance in inhibiting cellular lipid peroxidation.

The formation of Neutrophil Extracellular Traps (NETs) is the main mechanism by which Polymorphonuclear Neutrophils (PMNs) fight against microorganisms. Recent research suggests that the downregulation of GPX4 due to m6A modifications induced by METTL-3 leads to an increased accumulation of NETs in patients and mice suffering from sepsis-related ALI, alongside the occurrence of ferroptosis.[91]. Treatment of CLP mice with C23, a specific C1RP inhibitor, can help counteract the decrease in GPX4 and the rise in MDA levels in lung tissue. These results indicate that eC1RP triggers septic pulmonary ferroptosis by

reducing GPX4 levels and elevating lipid ROS concentrations. Therefore, regulating GPX4 by targeting eC1RP may offer a new therapeutic approach for sepsis and other inflammatory diseases [92]. Similarly, miR - 125b - 5p in ADSCs exosomes can alleviate inflammation - induced PMVECs ferroptosis by regulating the expression of Keap1/Nrf2/GPX4, thereby improving the acute lung injury in sepsis[93]. Protector Conjugate 1 (PCTR1) in tissue regeneration is an endogenous lipid mediator that has a protective effect on multi - organ damage. Inhibition of PCTR1 receptors ALX, PKA and CREB partially decreases GPX4 expression induced by PCTR1. Blocking CREB activity reverses the protective effects of PCTR1 on ferroptosis and lung injury[94]. Using the CLP model, researchers have demonstrated the involvement of both pyroptosis and ferroptosis in CLP-induced ALI. Additionally, it has been confirmed that GPX4 can reduce ROS levels, thereby modulating cell pyroptosis and ferroptosis to provide organ protection[95].

The absence of GPX4 intensifies lipid peroxidation and triggers ferroptosis. Miotto G, et al. discovered that LPS treatment leads to a reduction in GPX4 expression, which is restored by Fer-1[96]. This result was also validated by Zheng Xiao steam. Fer-1 increases the expression of GPX4 in high-fat diet-induced atherosclerotic mice[96], and it can regulate ferroptosis to protect the body from cardiac damage caused by sepsis[97, 98]. Fer-1 improves sepsis-induced cardiac dysfunction and reduces ferroptosis and inflammation in cardiac cells[99]. Similarly, the ATRA derivative ATPR enhances myocardial damage in septic mice by elevating the levels of glutathione (GSH) and malondialdehyde (MDA)[100]. YiQiFuMai Injection(YQFM) alleviates sepsis-induced cardiomyopathy(SIC) by boosting the xCT/GPX4 axis to decrease lipid peroxidation(LP) and prevent ferroptosis[101]. The GPX4 inhibitor can eliminate the protective effect of YQFM on LPS - stimulated H9c2 cells, indicating that YQFM primarily reduces myocardial cell damage by enhancing the xCT/GPX4 axis[101]. These studies play a leading role in guiding myocardial protection in sepsis and hold significant importance for clinically oriented practitioners.

Sepsis-associated encephalopathy (SAE) refers to a significant impairment of brain function caused by infections occurring outside the central nervous system that are linked to sepsis[102, 103]. Neuronal ferroptosis has been recognized as the primary contributor to cognitive decline and mortality in individuals experiencing sepsis-associated encephalopathy (SAE). Administering a low dose of acetaminophen (APAP) to septic mice has been found to effectively halt ferroptosis in the hippocampal tissue. This is achieved as APAP inhibits ferroptosis in the hippocampus of septic mice by targeting the GPX4 coupled with the FSP1 pathway[104, 105]. In the model of hemorrhagic stroke, selenium could reach the brain and stimulate the expression of the antioxidant GPX4, thereby safeguarding neurons[106]. Similarly, during the progression of sepsis, some patients may experience damage to the liver, kidney, and digestive system. Swift identification and prompt intervention can greatly enhance the chances of survival and overall outlook for critically ill patients. Nobiletin (NOB) is a plant-derived polymethoxyflavonoid and a promising protective agent against sepsis. Sepsis-induced acute liver injury (SALI) could be linked to the gut microbiota, where NOB shields against damage by decreasing ferroptosis and inflammation via the Nrf2-Gpx4 signaling pathway[107]. Additionally, YAP1, a pivotal regulator of the Hippo signaling

pathway, suppresses ferroptosis via ferritinophagy in liver cells, and its absence worsens sepsis-induced liver injury[108]. Acute kidney injury associated with sepsis (SA-AKI) is characterized by a high mortality rate and a lack of effective targeted treatments. Recent studies have found that dexmedetomidine reduces sepsis-induced kidney damage by increasing the binding of KEAP1 and HSP90 in the cytoplasm to decrease the degradation of GPX4 in the lipopolysaccharide-induced injury model in human and mouse renal tubular epithelial cells[109].

4. Conclusion

The rate of mortality from sepsis in the ICU is quite high. If early diagnosis and treatment are not attained, patients prognosis and quality of life will significantly decline. This article explores the role of programmed cell death in sepsis. GPX4 plays a crucial role in redox reactions and can control various cell death pathways. Consequently, it is significantly involved in sepsis treatment and may serve as a suitable target for drug regulation. Currently, research on GPX's role in rescuing sepsis primarily concentrates on ferroptosis and pyroptosis, with limited studies exploring how GPX4 regulates cuproptosis, necroptosis, autophagy and apoptosis to mitigate organ damage in sepsis. This also provides a direction for our next research and makes contributions to rescuing sepsis.

References

- [1] Singer, M et al The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama*, 2016. 315(8): p. 801-10.
- [2] Fleischmann, C et al Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. *Current Estimates and Limitations*. *Am J Respir Crit Care Med*, 2016. 193(3): p. 259-72.
- [3] Torres, L.K P. Pickkers, and T. van der Poll, Sepsis-Induced Immunosuppression. *Annu Rev Physiol*, 2022. 84: p. 157-181.
- [4] Shankar-Hari, M et al Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama*, 2016. 315(8): p. 775-87.
- [5] Delano, M.J. and P.A. Ward, Sepsis-induced immune dysfunction: can immune therapies reduce mortality? *J Clin Invest*, 2016. 126(1): p. 23-31.
- [6] Krysko, D.V et al Emerging role of damage-associated molecular patterns derived from mitochondria in inflammation. *Trends Immunol*, 2011. 32(4): p. 157-64.
- [7] Hotchkiss, R.S G. Monneret, and D. Payen, Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol*, 2013. 13(12): p. 862-74.
- [8] Jiang, Y et al Pyroptosis in septic lung injury: Interactions with other types of cell death. *Biomed Pharmacother*, 2023. 169: p. 115914.
- [9] Wu, D et al Epigenetic mechanisms of Immune remodeling in sepsis: targeting histone modification. *Cell Death Dis*, 2023. 14(2): p. 112.
- [10] Yang, Y.H J.F. Xie, and Y. Yang, [The role of programmed cell death protein 1/programmed cell death protein ligand 1 pathway in immune cell dysfunction in sepsis]. *Zhonghua Nei Ke Za Zhi*, 2021. 60(6): p. 571-574.
- [11] Flohé, L S. Toppo, and L. Orian, The glutathione peroxidase family: Discoveries and mechanism. *Free Radic Biol Med*, 2022. 187: p. 113-122.
- [12] Weaver, K. and R. Skouta, The Selenoprotein Glutathione Peroxidase 4: From Molecular Mechanisms to Novel Therapeutic Opportunities. *Biomedicines*, 2022. 10(4).
- [13] Mourio, M et al Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. *Biochim Biophys Acta*, 1982. 710(2): p. 197-211.
- [14] Stockwell, B.R J.P.F. Angeli, and H. Bayir, Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell*, 2017. 171(2): p. págs. 273-285.
- [15] Yang, W.S et al Regulation of ferroptotic cancer cell death by GPX4. *Cell*, 2014. 156(1-2): p. 317-331.
- [16] Angeli, J.P.F et al Ferroptosis Inhibition: Mechanisms and Opportunities. *Trends Pharmacol Sci*, 2017. 38(5): p. 489-498.
- [17] Dixon, S.J et al Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*, 2012. 149(5): p. 1060-72.
- [18] Stockwell, B.R et al Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell*, 2017. 171(2): p. 273-285.
- [19] Gaschler, M.M et al FINO(2) initiates ferroptosis through GPX4 inactivation and iron oxidation. *Nat Chem Biol*, 2018. 14(5): p. 507-515.
- [20] Doll, S et al ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol*, 2017. 13(1): p. 91-98.
- [21] Forcina, G.C. and S.J. Dixon, GPX4 at the Crossroads of Lipid Homeostasis and Ferroptosis. *Proteomics*, 2019. 19(18): p. e1800311.
- [22] Broz, P. and V.M. Dixit, Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol*, 2016. 16(7): p. 407-20.
- [23] Shi, J et al Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature*, 2015. 526(7575): p. 660-5.
- [24] Wei, X et al Role of pyroptosis in inflammation and cancer. *Cell Mol Immunol*, 2022. 19(9): p. 971-992.
- [25] Kanneganti, T.D et al Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. *J Biol Chem*, 2006. 281(48): p. 36560-8.
- [26] Sutterwala, F.S et al Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. *Immunity*, 2006. 24(3): p. 317-27.
- [27] Martinon, F K. Burns, and J. Tschopp, The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell*, 2002. 10(2): p. 417-26.
- [28] Kayagaki, N et al Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature*, 2015. 526(7575): p. 666-71.
- [29] Evavold, C.L et al The Pore-Forming Protein Gasdermin D Regulates Interleukin-1 Secretion from Living Macrophages. *Immunity*, 2018. 48(1): p. 35-44.e6.
- [30] Hagar, J.A et al Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. *Science*, 2013. 341(6151): p. 1250-3.
- [31] Kayagaki, N et al Non-canonical inflammasome activation targets caspase-11. *Nature*, 2011. 479(7371): p. 117-21.

- [32] He, Y et al NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. *Nature*, 2016. 530(7590): p. 354-7.
- [33] Tang, D et al Ferroptosis: molecular mechanisms and health implications. *Cell Res*, 2021. 31(2): p. 107-125.
- [34] Galluzzi, L et al Necroptosis: Mechanisms and Relevance to Disease. *Annu Rev Pathol*, 2017. 12: p. 103-130.
- [35] Chen, R R. Kang, and D. Tang, The mechanism of HMGB1 secretion and release. *Exp Mol Med*, 2022. 54(2): p. 91-102.
- [36] Pasparakis, M. and P. Vandenabeele, Necroptosis and its role in inflammation. *Nature*, 2015. 517(7534): p. 311-20.
- [37] Riebeling, T U. Kunzendorf, and S. Krautwald, The role of RHIM in necroptosis. *Biochem Soc Trans*, 2022. 50(4): p. 1197-1205.
- [38] Annibaldi, A. and P. Meier, Checkpoints in TNF-Induced Cell Death: Implications in Inflammation and Cancer. *Trends Mol Med*, 2018. 24(1): p. 49-65.
- [39] Dillon, C.P et al Survival function of the FADD-CASPASE-8-cFLIP(L) complex. *Cell Rep*, 2012. 1(5): p. 401-7.
- [40] Pajuelo, D et al NAD(+) Depletion Triggers Macrophage Necroptosis, a Cell Death Pathway Exploited by *Mycobacterium tuberculosis*. *Cell Rep*, 2018. 24(2): p. 429-440.
- [41] Flores-Romero, H U. Ros, and A.J. Garcia-Saez, Pore formation in regulated cell death. *Embo j*, 2020. 39(23): p. e105753.
- [42] Sun, L et al Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell*, 2012. 148(1-2): p. 213-27.
- [43] Janko, C et al Redox modulation of HMGB1-related signaling. *Antioxid Redox Signal*, 2014. 20(7): p. 1075-85.
- [44] Canli, Ö et al Glutathione peroxidase 4 prevents necroptosis in mouse erythroid precursors. *Blood*, 2016. 127(1): p. 139-48.
- [45] Hu, Q et al GPX4 and vitamin E cooperatively protect hematopoietic stem and progenitor cells from lipid peroxidation and ferroptosis. *Cell Death Dis*, 2021. 12(7): p. 706.
- [46] Dannappel, M et al RIPK1 maintains epithelial homeostasis by inhibiting apoptosis and necroptosis. *Nature*, 2014. 513(7516): p. 90-4.
- [47] Takahashi, N et al RIPK1 ensures intestinal homeostasis by protecting the epithelium against apoptosis. *Nature*, 2014. 513(7516): p. 95-9.
- [48] Rickard, J.A et al RIPK1 regulates RIPK3-MLKL-driven systemic inflammation and emergency hematopoiesis. *Cell*, 2014. 157(5): p. 1175-88.
- [49] Ding, Y et al Identification of a small molecule as inducer of ferroptosis and apoptosis through ubiquitination of GPX4 in triple negative breast cancer cells. *J Hematol Oncol*, 2021. 14(1): p. 19.
- [50] Meissner, F K. Molawi, and A. Zychlinsky, Superoxide dismutase 1 regulates caspase-1 and endotoxic shock. *Nat Immunol*, 2008. 9(8): p. 866-72.
- [51] Pan, S. and B.C. Berk, Glutathiolation regulates tumor necrosis factor-alpha-induced caspase-3 cleavage and apoptosis: key role for glutaredoxin in the death pathway. *Circ Res*, 2007. 100(2): p. 213-9.
- [52] Goossens, V et al Redox regulation of TNF signaling. *Biofactors*, 1999. 10(2-3): p. 145-56.
- [53] Kang, S.J et al Distinct downstream pathways of caspase-11 in regulating apoptosis and cytokine maturation during septic shock response. *Cell Death Differ*, 2002. 9(10): p. 1115-25.
- [54] Gon, S T. Gatanaga, and F. Sendo, Involvement of two types of TNF receptor in TNF-alpha induced neutrophil apoptosis. *Microbiol Immunol*, 1996. 40(6): p. 463-5.
- [55] Pennica, D et al Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature*, 1984. 312(5996): p. 724-9.
- [56] Itoh, N et al The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell*, 1991. 66(2): p. 233-43.
- [57] Pan, G et al The receptor for the cytotoxic ligand TRAIL. *Science*, 1997. 276(5309): p. 111-3.
- [58] Srinivasula, S.M et al Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Mol Cell*, 1998. 1(7): p. 949-57.
- [59] Li, P et al Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell*, 1997. 91(4): p. 479-89.
- [60] Bratton, S.B et al Recruitment, activation and retention of caspases-9 and -3 by Apaf-1 apoptosome and associated XIAP complexes. *Embo j*, 2001. 20(5): p. 998-1009.
- [61] Nicholson, D.W. and N.A. Thornberry, Caspases: killer proteases. *Trends Biochem Sci*, 1997. 22(8): p. 299-306.
- [62] Liu, X et al Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell*, 1996. 86(1): p. 147-57.
- [63] Sheng, S.Y et al Regulated cell death pathways in cardiomyopathy. *Acta Pharmacol Sin*, 2023. 44(8): p. 1521-1535.
- [64] Yao, L et al Plumbagin is a novel GPX4 protein degrader that induces apoptosis in hepatocellular carcinoma cells. *Free Radic Biol Med*, 2023. 203: p. 1-10.
- [65] Yan, X et al Photodynamic therapy inhibits cancer progression and induces ferroptosis and apoptosis by targeting P53/GPX4/SLC7A11 signaling pathways in cholangiocarcinoma. *Photodiagnosis Photodyn Ther*, 2024. 47: p. 104104.
- [66] Mizushima, N. and M. Komatsu, Autophagy: renovation of cells and tissues. *Cell*, 2011. 147(4): p. 728-41.
- [67] Mizushima, N et al Autophagy fights disease through cellular self-digestion. *Nature*, 2008. 451(7182): p. 1069-75.
- [68] Klionsky, D.J The molecular machinery of autophagy: unanswered questions. *J Cell Sci*, 2005. 118(Pt 1): p. 7-18.
- [69] Chen, F et al Autophagy-Dependent Ferroptosis in Cancer. *Antioxid Redox Signal*, 2023. 39(1-3): p. 79-101.
- [70] Li, X S. He, and B. Ma, Autophagy and autophagy-related proteins in cancer. *Mol Cancer*, 2020. 19(1): p. 12.
- [71] Chen, X et al Identification of HPCAL1 as a specific autophagy receptor involved in ferroptosis. *Autophagy*, 2023. 19(1): p. 54-74.
- [72] Wu, Z et al Chaperone-mediated autophagy is involved in the execution of ferroptosis. *Proc Natl Acad Sci U S A*, 2019. 116(8): p. 2996-3005.
- [73] Wu, K et al Creatine kinase B suppresses ferroptosis by phosphorylating GPX4 through a moonlighting function. *Nat Cell Biol*, 2023. 25(5): p. 714-725.
- [74] Rubio, N et al Spatiotemporal autophagic degradation of oxidatively damaged organelles after photodynamic stress is amplified by mitochondrial reactive oxygen species. *Autophagy*, 2012. 8(9): p. 1312-24.
- [75] Jiang, W et al L-carnitine supplementation during in vitro culture regulates oxidative stress in embryos from bovine aged oocytes. *Theriogenology*, 2020. 143: p. 64-73.

- [76] Morgan, A.H et al A novel role for 12/15-lipoxygenase in regulating autophagy. *Redox Biol*, 2015. 4: p. 40-7.
- [77] Kahlson, M.A. and S.J. Dixon, Copper-induced cell death. *Science*, 2022. 375(6586): p. 1231-1232.
- [78] Tsvetkov, P et al Copper induces cell death by targeting lipoylated TCA cycle proteins. *Science*, 2022. 375(6586): p. 1254-1261.
- [79] Tsvetkov, P et al Mitochondrial metabolism promotes adaptation to proteotoxic stress. *Nat Chem Biol*, 2019. 15(7): p. 681-689.
- [80] Tardito, S et al Copper-dependent cytotoxicity of 8-hydroxyquinoline derivatives correlates with their hydrophobicity and does not require caspase activation. *J Med Chem*, 2012. 55(23): p. 10448-59.
- [81] Gupta, G. and B. Fadeel, ToxPoint: Copper Is the New Showstopper. *Toxicol Sci*, 2022. 189(1): p. 3-4.
- [82] Nagai, M et al The oncology drug elesclomol selectively transports copper to the mitochondria to induce oxidative stress in cancer cells. *Free Radic Biol Med*, 2012. 52(10): p. 2142-50.
- [83] Członkowska, A et al Wilson disease. *Nat Rev Dis Primers*, 2018. 4(1): p. 21.
- [84] Xu, J et al Multi-omics pan-cancer study of cuproptosis core gene FDX1 and its role in kidney renal clear cell carcinoma. *Front Immunol*, 2022. 13: p. 981764.
- [85] Li, H et al GSH exhaustion via inhibition of xCT-GSH-GPX4 pathway synergistically enhanced DSF/Cu-induced cuproptosis in myelodysplastic syndromes. *Free Radic Biol Med*, 2024. 222: p. 130-148.
- [86] Mao, C et al DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. *Nature*, 2021. 593(7860): p. 586-590.
- [87] Liu, T et al Ferrous-Supply-Regeneration Nanoengineering for Cancer-Cell-Specific Ferroptosis in Combination with Imaging-Guided Photodynamic Therapy. *ACS Nano*, 2018. 12(12): p. 12181-12192.
- [88] Zhou, J et al Photothermally Triggered Copper Payload Release for Cuproptosis-Promoted Cancer Synergistic Therapy. *Angew Chem Int Ed Engl*, 2023. 62(12): p. e202213922.
- [89] Chen, L J. Min, and F. Wang, Copper homeostasis and cuproptosis in health and disease. *Signal Transduct Target Ther*, 2022. 7(1): p. 378.
- [90] Evans, L et al Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021. *Crit Care Med*, 2021. 49(11): p. e1063-e1143.
- [91] van der Poll, T et al The immunopathology of sepsis and potential therapeutic targets. *Nat Rev Immunol*, 2017. 17(7): p. 407-420.
- [92] Xie, J et al The Epidemiology of Sepsis in Chinese ICUs: A National Cross-Sectional Survey. *Crit Care Med*, 2020. 48(3): p. e209-e218.
- [93] Zhang, H et al Neutrophil extracellular traps mediate m(6)A modification and regulates sepsis-associated acute lung injury by activating ferroptosis in alveolar epithelial cells. *Int J Biol Sci*, 2022. 18(8): p. 3337-3357.
- [94] Shimizu, J et al Extracellular CIRP Promotes GPX4-Mediated Ferroptosis in Sepsis. *Front Immunol*, 2022. 13: p. 903859.
- [95] Shen, K et al miR-125b-5p in adipose derived stem cells exosome alleviates pulmonary microvascular endothelial cells ferroptosis via Keap1/Nrf2/GPX4 in sepsis lung injury. *Redox Biol*, 2023. 62: p. 102655.
- [96] Lv, Y et al Protectin conjugates in tissue regeneration 1 alleviates sepsis-induced acute lung injury by inhibiting ferroptosis. *J Transl Med*, 2023. 21(1): p. 293.
- [97] Cao, Z et al Crosstalk of pyroptosis, ferroptosis, and mitochondrial aldehyde dehydrogenase 2-related mechanisms in sepsis-induced lung injury in a mouse model. *Bioengineered*, 2022. 13(3): p. 4810-4820.
- [98] Miotto, G et al Insight into the mechanism of ferroptosis inhibition by ferrostatin-1. *Redox Biol*, 2020. 28: p. 101328.
- [99] Xiao, Z et al Ferrostatin-1 alleviates lipopolysaccharide-induced cardiac dysfunction. *Bioengineered*, 2021. 12(2): p. 9367-9376.
- [100] Dan, Z et al 4-amino-2-trifluoromethyl-phenyl retinate alleviates lipopolysaccharide-induced acute myocardial injury through activation of the KLF4/p62 axis. *Cell Signal*, 2024. 114: p. 111001.
- [101] Guo, L et al YIQIFUMAI INJECTION AMELIORATED SEPSIS-INDUCED CARDIOMYOPATHY BY INHIBITION OF FERROPTOSIS VIA XCT/GPX4 AXIS. *Shock*, 2024. 61(4): p. 638-645.
- [102] Sonnevile, R et al Potentially modifiable factors contributing to sepsis-associated encephalopathy. *Intensive Care Med*, 2017. 43(8): p. 1075-1084.
- [103] Chen, J et al A retrospective study of sepsis-associated encephalopathy: epidemiology, clinical features and adverse outcomes. *BMC Emerg Med*, 2020. 20(1): p. 77.
- [104] Chu, J et al Acetaminophen impairs ferroptosis in the hippocampus of septic mice by regulating glutathione peroxidase 4 and ferroptosis suppressor protein 1 pathways. *Brain Behav*, 2023. 13(8): p. e3145.
- [105] Chu, J et al Acetaminophen alleviates ferroptosis in mice with sepsis-associated encephalopathy via the GPX4 pathway. *Hum Exp Toxicol*, 2022. 41: p. 9603271221133547.
- [106] Alim, I et al Selenium Drives a Transcriptional Adaptive Program to Block Ferroptosis and Treat Stroke. *Cell*, 2019. 177(5): p. 1262-1279.e25.
- [107] Huang, W et al Nobiletin protects against ferroptosis to alleviate sepsis-associated acute liver injury by modulating the gut microbiota. *Food Funct*, 2023. 14(16): p. 7692-7704.
- [108] Wang, J et al YAP1 protects against septic liver injury via ferroptosis resistance. *Cell Biosci*, 2022. 12(1): p. 163.
- [109] Li, J et al Dexmedetomidine alleviates renal tubular ferroptosis in sepsis-associated AKI by KEAP1 regulating the degradation of GPX4. *Eur J Pharmacol*, 2023. 961: p. 176194.