SIRT1-mediated p53 deacetylation inhibits ferroptosis and alleviates heat stress-induced lung epithelial cells injury

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ABSTRACT

Objective: Acute lung injury (ALI) is a common complication of heat stroke (HS) and a direct cause of death. However, the mechanism underlying ALI following HS remains unclear.

Method: To investigate whether ferroptosis is involved in HS-ALI. We established a HS model of mice and mouse lung epithelial-2 cells (MLE-2). The severity of lung injury was measured by H&E staining, the wet-to-dry lung weight ratio, and Transmission electron microscopy. Potential markers of ferroptosis Fe2+, malondialdehyde (MDA), hydroxynonenal (4-HNE) and lipid peroxidation were detected. The percentages of cell death and viability induced by HS were assessed by LDH and CCK8 assays. SLC7A11, ACSL4, GPX4, SIRT1, p53, and p53 K382 acetylation levels were measured by Western blot.

Results: The administration of ferroptosis inhibitor ferrostatin-1 (Fer-1) could significantly ameliorate lung injury, inhibiting levels of MDA and 4-HNE, and ameliorating HS-induced increased ACSL4, decreased SLC7A11 and GPX4, suggesting ferroptosis was involved in HS-induced ALI in vivo and in vitro. Moreover, SIRT1 expression decreased, and p53 K382 acetylation levels increased in MLE-2 cells. Activation of SIRT1 could improve lung epithelial ferroptosis caused by HS in vivo and in vitro. Besides, the activation of SIRT1 could significantly reduce the p53 K382 acetylation levels, suggesting that activation of SIRT1 could prevent ferroptosis via inhibiting p53 acetylation.

Conclusion: These findings substantiate the vital role of the SIRT1/p53 axis in mediating ferroptosis in HS-ALI, suggesting that targeting SIRT1 may represent a novel therapeutic strategy to ameliorate ALI during HS.

1. Introduction

The global incidence of heat stroke (HS) has increased in recent years and is associated with high morbidity and mortality [1]. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are widely acknowledged as common complications in critically ill patients, responsible for significant morbidity, mortality, and related healthcare costs [2]. It is well-recognized that ALI/ARDS is a common complication of heat stroke [3]. Therefore, the precise roles of lung epithelial ferroptosis in ALI induced by HS remains to be determined.

Ferroptosis is a new category of programmed cell death triggered by iron-dependent lipid peroxide accumulation, which differs from other cell death types, such as apoptosis, necroptosis, and autophagy [8]. Tumor suppressor p53 is important to cell fate decision under different stimuli, also functioning in DNA repair, cell cycle arrest, and cell death. p53 is subject to a wide range of post-translational modifications (PTM) of p53 protein, including acetylation, ubiquitination, phosphorylation, and sumoylation [9]. Among these PTMs, p53 acetylation has attracted extensive attention, and is essential for regulation of p53 activity and cell fate determination [10]. Growing evidence have shown that inhibition of p53 could alleviate intestinal ischemia/reperfusion-induced acute lung injury by inhibiting ferroptosis, suggesting that p53 acts as a positive ferroptosis regulator [11]. p53 acetylation plays a pivotal role in p53-mediated ferroptosis in tumor suppression [9,12]. In addition, p53 also plays a crucial development following exertional heat stroke (EHS) [7].
role in the HS-induced mitochondrial apoptosis in endothelial cells [13]. However, the role of p53 acetylation in ferroptosis during HS-ALI remains largely unknown.

Silent information regulator 1 (SIRT1), the human ortholog of yeast silence information regulator 2 (SIRT2), is a component of mammalian NAD-dependent sirtuin deacetylases, which catalyze the process of histone deacetylation together with additional substrates and influence several processes such as inflammation, senescence, mitochondrial biogenesis, cell senescence, apoptosis, and circadian rhythms [14]. It has been found that p53 deacetylation induced by SIRT1 may promote renal tubular epithelial cell autophagy and attenuate sepsis-induced acute kidney injury (SAKI) [15]. In recent studies, SIRT1 could inhibit ferroptosis-induced myocardial cell death through the p53/SLC7A11 axis in myocardial ischemia-reperfusion injury [16]. Thus, we proposed a hypothesis that induction of p53 deacetylation, by SIRT1 activation could inhibit ferroptosis induced by HS.

In our study, we investigated that ferroptosis was involved in HS-induced ALI in an HS animal model and a cellular model of heat stress. Furthermore, SIRT1/p53 axis may play pivotal roles in regulating lung epithelial ferroptosis in HS-induced ALI. Our study aims to investigate the role of SIRT1-mediated p53 deacetylation in HS-induced ALI, hoping to highlight a promising and targeting approach to heat stroke treatment.

2. Materials and methods

2.1. Materials

C57BL/6 mice (aged 10 to 12 weeks) used in this study were obtained from the Experimental Animal Center of Southern Medical University. All mice were housed under a controlled 12/12-h light/dark cycle at a constant temperature (24 ± 1 °C) and (54 ± 2%) relative humidity with free access to a pelleted rodent diet and water. All protocols of animal experiments followed the guidelines approved by the Chinese Association of Laboratory Animal Care and were approved by the Ethical Committee for Animal Experimentation of Nanfang Hospital.

2.2. Hs protocol for animals

HS-ALI models were induced in mice as previously described in our reported method [17]. Animals were placed in a climate chamber that was maintained at a constant temperature of 39.5 ± 0.2 °C with 60 ± 5% relative humidity in the absence of food or water, and rectal temperature (Tc) was measured at intervals of ten minutes. The time point at which the Tc reached 42.5 °C was used as a reference point of HS onset. All mice were then returned to their original cages in an environment at 25 °C with water after HS. The control group underwent the same procedure without HS treatment. Chemical reagents at doses of 10 mg/kg for ferrostatin-1 (Fer-1) were intraperitoneally injected 2 h before HS as required. All groups of mice were sacrificed at time points as indicated after 1.0% pentobarbital sodium (5 mg/100 g BW, i.p.) administration to harvest the lung tissues.

2.3. Transmission electron microscopy

Fresh lung tissues of 1 mm³ were harvested and then fixed in 2% formaldehyde and 2.5% glutaraldehyde. The tissue blocks were then fixed in 1% osmic acid, dehydrated with ethanol and acetone gradients, and embedded in epoxy resin and propylene oxide overnight. Samples were then cut into 70-nm-thick ultrathin sections. The sections were then observed under an H-7650 transmission electron microscope (Hitachi, Tokyo, Japan) at low magnification (×8000) and then analyzed at high magnification (×40000) to observe the mitochondrion. Two professional pathologists analyzed the images in a blinded manner.

2.4. Histopathological analysis

Lung samples were fixed in 10% formalin and then embedded in paraffin. Tissue blocks were cut into 5-μm slices, stained with hematoxylin and eosin (H&E), and analyzed under a light microscope. Lung injury was analyzed by an experienced investigator blinded to the level of induced injury (absent, mild, moderate, or severe; score 0–3) on the basis of the presence of exudates, hyperemia and congestion, neutrophilic infiltrates, intra-alveolar hemorrhage and debris, and cellular hyperplasia. In brief, edema, atelectasis, necrosis, alveolar and interstitial inflammation, and hemorrhage, and hyaline membrane formation were each scored on a scale of 0–4; 0, no injury; 1, 25% injury; 2, 50% injury; 3, 75% injury; and 4, 100% injury. Each injury was scored in ten randomly selected fields (200×) from each slide.

2.5. Fe²⁺, malondialdehyde (MDA), hydroxynonenal (4-HNE) and lipid peroxidation assays

The relative iron concentration in lung tissue lysates was assessed with an Iron Assay Kit (ab83366; Abcam). The relative concentration of MDA in lung tissue lysates and cell lysates was assessed with a Lipid Peroxidation Assay Kit (ab118970; Abcam). Immunohistochemical staining with anti-4-HNE antibody according to the manufacturers' instructions. The lipid peroxidation assays were stained with 2 μM C11-BODIPY (581/591) probe (Invitrogen, USA) in accordance with the manufacturer's instructions. Cells were visualized under Olympus BX63 fluorescence microscope and then analyzed by ImageJ software (NIH, Bethesda, MD, USA). Oxidized BODIPY (O-BODIPY) and reduced BODIPY (R-BODIPY) were observed at excitation/emission wavelengths of 488/510 nm (traditional FITC filter set) and 581/591 nm (Texas Red1 filter set).

2.6. Cell culture, treatment, and transfection with adenovirus

MLE-2 cells were purchased from the ATCC (Manassas, VA, USA) and cultured in RPMI 1640 medium (Invitrogen) supplemented with 5% heat-inactivated fetal bovine serum, L-glutamine, penicillin, and streptomycin at 37 °C and 5% CO₂ in humidified air. For HS treatment in vitro, the culture medium was replaced with fresh medium, and then cells were placed
in an incubator containing 5% CO₂ at 42 ± 0.5°C for 3 h. Subsequently, the cells were incubated in a normal incubator at 37°C and 5% CO₂. For treatment with Fer-1, cells were pretreated with the different dose of Fer-1 (0.1, 0.5, 1.0 or 10 µM) 1 h before HS treatment. The adenoviral vector expressing Flag- and GFP-tagged Ad-p53K382R and Ad-SIRT1 was provided by Gene Pharma (Shanghai, China).

2.7. Western blot analysis
For whole-cell lysates, lung tissues and MLE-2 cells were harvested using radioimmunoprecipitation assay (RIPA) lysis buffer containing 1× protease inhibitor cocktail. After separation by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes. Subsequently, the membranes were blocked in 5% bovine serum albumin (BSA) at room temperature (RT) for 1 h followed by immunoblotting at 4°C overnight with primary antibodies. The membranes were then incubated with secondary antibodies for one hour. Target protein was detected using enhanced chemiluminescence reagents. The primary antibodies were as follows: anti-SIRT1 (ab110304, Abcam), anti-SLC7A11 (PA1-16893, Invitrogen), anti-ACSL4 (A6826, Abclonal), anti-GPX4 (ab125066, Abcam), anti-p53 (60283-2, Proteintech), anti-ack382-p53 (#2525, Cell Signaling Technology), anti-GAPDH (AC002, Abclonal). The secondary antibodies were horseradish peroxidase-labeled goat anti-mouse (AS014, Abclonal) and anti-rabbit (AS003, Abclonal) antibodies. Quantification was performed by measuring the density of blot bands using ImageJ software. Protein expression levels were normalized relative to the levels of GAPDH.

2.8. Cell viability quantification and Lactase dehydrogenase release assay
Cell Counting Kit-8 (CCK-8) (Sigma, Saint Louis, USA) reagent was used to examine cell viability according to the manufacturer’s instructions. The extent of cellular injury was determined by lactate dehydrogenase (LDH) leakage using LDH cytotoxicity detection kit (Dojindo Laboratory, Kumamoto, Japan). Absorbance at 450 nm and 490 nm was detected by a plate reader (BioRad, Hercules, CA, USA).

2.9. Statistical analysis
Results are expressed as the mean ± standard deviation. Student’s t test was used to analyze differences between two groups. Statistical comparisons of three or more groups were performed using one-way analysis of variance (ANOVA), followed by post hoc analysis. Quantitative data are from at least three separate experiments performed in duplicate. All tests for statistical significance were performed using GraphPad software (La Jolla, CA). p values < .05 were considered statistically significant.

3. Results
3.1. Heat stroke induces Ferroptosis in lung tissue
To investigate the mechanism of ALI development following HS, we established a murine model of HS. First, we measured the severity of lung injury at 12 h and 24 h after HS (Figure 1(A–B)). Lung injury was mainly presented as dema, atelectasis, necrosis, alveolar and interstitial inflammation, hemorrhage, and hyaline membrane formation by H&E staining. Meanwhile, the wet-to-dry lung weight ratio significantly increased. One recent study has shown that ferroptosis plays a key role in rhabdomyolysis (RM) induced by exertional heat stroke (EHS) [7]. Accordingly, we analyzed the expression of ferroptosis-related proteins in the lung tissue following HS to further investigate whether ferroptosis is involved in HS-induced ALI. Western blot results showed that ACSL4 levels significantly increased at 12 h and 24 h following HS, SLC7A11 and GPX4 levels decreased compared with the control group (Figure 1(C)). Potential markers of ferroptosis Fe²⁺ and MDA were significantly increased in lung tissue after HS (Figure 1(D)). Consistent with previous studies, Figure 1(E) showed these lung epithelial cells injury mainly presented as rupture of the outer mitochondrial membrane and disappearance of mitochondrial cristae after HS.

3.2. Ferroptosis contributes to acute lung injury development following heat stroke
To determine the pathogenic role of ferroptosis in acute lung injury development following HS, ferroptosis inhibitor ferrostatin-1 (Fer-1) was pretreated before HS. H&E staining showed that pretreatment with Fer-1 significantly inhibited lung injury and wet-to-dry lung weight ratio following HS (Figure 2(A–B)). Next, these results suggested that pretreatment with Fer-1 significantly attenuated MDA and 4-HNE levels following HS (Figure 2(C–D)). Furthermore, inhibition of ferroptosis using Fer-1 significantly downregulated ACSL4, upregulated SLC7A11 and GPX4 protein expression in lung tissues (Figure 2(E)). Overall, these results indicate that ferroptosis is involved in HS-induced ALI.

3.3. Heat stress induced Ferroptosis in MLE-12 cells
To further investigate the role of ferroptosis induced by HS in vitro, the MLE-12 cells were exposed to heat stress for 3 h, followed by further incubation for 0, 6, 12, and 24 h. The CCK8 assay showed that viability of MLE-12 cells gradually decreased at a time-manner, suggesting the occurrence of cell injury by heat stress (Figure 3(A)). Immunoblot analysis results suggested ACSL4 gradually increased time-dependently and SLC7A11 and GPX4 gradually decreased at 6 h and lasted until 24 h, suggesting that ferroptosis was induced in HS-treated MLE-2 cells (Figure 3(B)). To further confirm ferroptosis induction, the levels of MDA and Lipid ROS were detected. These results revealed heat stress could induce upregulation of MDA and lipid ROS in a time-dependent manner (Figure 3(C–D)). Furthermore, MLE-2 cells were pre-treated with different doses of Fer-1(0.1, 0.5, 1.0, or 10 µM)
for 1 h and then exposed to heat stress. Our analysis revealed that 1 μM Fer-1 significantly improved cell viability during HS (Figure 3(E)). Pretreatment with Fer-1 protected against MLE-2 cell death induced by HS, with decreased LDH release (Figure 3(F)), reduced levels of lipid peroxidation product MDA (Figure 3(G)), and inhibited lipid peroxidation as indicated by C11 BODIPY 581/591 fluorescence (Figure 3(H)). In addition, Fer-1 treatment significantly ameliorated heat stress-induced increase in ACSL4 and decrease in SLC7A11 and GPX4. Therefore, our data suggest that Fer-1 inhibits heat stress-induced ferroptosis.

3.4. Role of SIRT1 in activating heat stress-induced ferroptosis in MLE-12 cells

It has been well recognized that SIRT1 can protect mice against alcohol-induced liver injury by reducing ferroptosis [18].

Figure 1. Ferroptosis induced by HS in lung tissue. All samples of lung tissues were collected at 12 h and 24 h after HS. Sham mice were included as controls. (A) Representative H&E staining of lung tissue and pathological score (Control, HS-R12h, and HS-R24h, scale bar = 50 μm in H&E). (B) Representative wet-to-dry lung weight ratio (Control, HS-R12h, and HS-R24h). (C) Western blotting analysis and quantification of ferroptosis-related proteins, including SLC7A11, ACSL4 and GPX4 in the lung tissue. (D) The levels of Fe²⁺ and MDA were assayed. (E) Representative TEM images of mitochondrial morphology in lung epithelial cells after HS. Red arrows: mitochondria. Scale bar: 500 nm. Data are shown as the mean ± SD. n = 4. *p < .05, **p < .01.
Decreased SIRT1 expression was observed in MLE-12 cells following HS induction (Figure 4(A)). To investigate the role of SIRT1 in heat stress-induced cell ferroptosis, MLE-12 cells were transfected with a plasmid expressing SIRT1. Overexpression of SIRT1 was confirmed by Western blot assays (Figure 4(B)). Treatment with the SIRT1 plasmid significantly reversed HS-induced SLC7A11 and GPX4 downregulation, ACSL4 upregulation, and alleviated the HS-induced cell death with decreased LDH release (Figure 4(C–D)). Furthermore, SIRT1 overexpressing plasmid significantly inhibited lipid peroxidation and diminished MDA levels (Figure 4(E–F)). Taken together, these results suggest that SIRT1 play an important role in regulating heat stress-induced ferroptosis in MLE-12 cells.

3.5. Decreased p53 acetylation inhibits ferroptosis-induced MLE-12 cell death

It has well been reported that p53 acetylation modification is crucial for its activity [19]. To address the role of p53 acetylation in MLE-12 cell ferroptosis induced by heat stress, we quantified p53 K382 acetylation and p53 protein levels in MLE-12 cells. We found that p53 K382 acetylation and p53 protein levels increased following heat stress time-dependently (Figure 5(A)). After MLE-12 cells were transfected with adenovirus expressing p53K382R, cellular immunofluorescence showed MLE-12 cells were well transfected with adenovirus expressing p53K382R (Figure 5(B)). Meanwhile, the adenovirus expressing p53K382R significantly downregulated the p53 acetylation at K382 and upregulated the expression of p53 protein (Figure 5(C)). These results suggested adenovirus expressing p53K382R could successfully downregulate the p53 K382 acetylation. Treatment with the adenovirus expressing p53K382R alleviated HS-induced cell death, with decreased LDH release and MDA levels (Figure 5(D–E)). Furthermore, treatment with the adenovirus expressing p53K382R could significantly increase HS-induced SLC7A11 and GPX4 downregulation and inhibit ACSL4 expression (Figure 5(F)).
3.6. Activation of SIRT1 decreases p53 K382 acetylation levels in MLE-2 cells

SIRT1 is well-recognized as a NAD$^+$-dependent deacetylase to directly deacetylate p53 and mediate its function [20]. Interestingly, growing evidence suggest that SIRT1 deacetylates the Lys382 residue of p53 [21]. Based on these aforementioned studies, we hypothesized that SIRT1 might deacetylate p53 (K382) in HS-ALI. Accordingly, we explored whether SIRT1 regulates p53 K382 acetylation in MEL-2 cells, the plasmid overexpressing SIRT1 were used. Western blot results SIRT1 overexpressing plasmid significantly decreased HS-induced p53 K382 acetylation but did not affect p53 protein expression (Figure 6). These results suggest that activation of SIRT1 could significantly reduce the level of p53 K382 acetylation in MLE-2 cells.

4. Discussion

In this report, we corroborated that ferroptosis was involved in HS-induced ALI in vivo and in vitro. Furthermore, we found that increased p53 acetylation levels promote lung epithelial ferroptosis in HS-induced ALI. The plasmid overexpressing SIRT1 could reduce p53 acetylation levels and inhibit HS-induced MLE-2 cells ferroptosis, suggesting the deacetylation of p53 mediated by SIRT1 can reverse this process to attenuate ferroptosis (Figure 7). Importantly, SIRT1/p53 axis may play vital roles in mediating lung epithelial ferroptosis in HS-ALI. These findings suggest a new perspective for elucidating the underlying mechanisms of HS-ALI, providing some therapeutic strategies for targeting SIRT1 for future HS-ALI treatment.

In recent years, ferroptosis has emerged as a new form of cell death implicated in various human diseases [22]. One recent study has shown that ferroptosis plays a key role in rhabdomyolysis induced by exertional heat stroke [7]. Consistent with previous literature, we also found that ferroptosis contributed to HS-induced ALI. SLC7A11, a heterodimer of the light chain subunit, is component of System Xc-, a cell membrane cystineglutamate antiporterin the System Xc- GSH-GPX4 pathway, which is the main prevention system for ferroptosis [16]. GPX4 is well-established to be an essential regulator of ferroptosis, which insufficiency may lead to increase levels of uncontrolled lipid peroxidation, culminating in ferroptosis. The current study suggests a key pathologic role of ACSL4 in mediating EHS-induced skeletal muscle cell ferroptosis activation via lipid peroxidation [23]. In addition, pretreatment with the ferroptosis inhibitor Fer-1 could markedly inhibit the levels of MDA and 4-HNE, ameliorate HS-induced increase in ACSL4 and decrease in SLC7A11 and GPX4, and attenuate HS-ALI, suggesting that inhibiting ferroptosis can significantly ameliorate HS-induced ALI.
SIRT1, the most extensive studied sirtuins family member, has been widely reported to play a protective role in various biologic systems including nutrient starvation, DNA repair, aging, and oxidative stress [24]. In the present study, SIRT1 protein levels decreased in HS-induced MLE-2 cells, overexpression of SIRT1 reduced ferroptosis-induced cell death following HS. Moreover, a plasmid overexpressing SIRT1 could significantly inhibit HS-induced MLE-2 cells ferroptosis, as illustrated by downregulation of lipid peroxidation and MDA levels, increase HS-induced SLC7A11 and GPX4, and decrease ACSL4 expression. p53 mainly functions as a transcription factor which is functionally mediated by several post-translational modifications [25]. Specifically, p53 acetylation at many positions play an important role in p53 transcriptional activity and cell fate determination [26]. Lysine K370/372/373R and K381/382/386R mutants of p53 decreased its stability partially due to their regulatory effects on p53 transcriptional activity [27]. Interestingly, growing evidence suggest that SIRT1 deacetylates the Lys382 residue of p53 in various physiological conditions [28,29]. A previous study indicates that SIRT1 knockdown can decrease acetylation of p53 (K382) in Vitamin D receptor (VDR) deficient podocytes [30]. Accordingly, our results suggested p53 K382 acetylation and p53 protein levels increased following heat stress time-dependently. Meanwhile, SIRT1 activation significantly decreased HS-induced p53 K382 acetylation, but did not change p53 protein expression in vitro. In addition, treatment with the p53K382R mutant adenovirus expressing p53K382R could significantly inhibit lung epithelial ferroptosis, shown by increase HS-induced SLC7A11 and GPX4 and decrease ACSL4 expression. These results suggest that activation of SIRT1 can significantly reduce lung epithelial ferroptosis and levels of p53 acetylation in HS-ALI.

5. Conclusion
To summarize, our study reveals that ferroptosis was involved in HS-induced ALI in vivo and in vitro. Moreover, SIRT1 overexpression reduced p53 acetylation levels and inhibit HS-induced MLE-2 cells ferroptosis, suggesting the deacetylation of p53 mediated by SIRT1 can reverse this process. Importantly, these findings suggest a SIRT1/p53 axis...
Figure 5. Decreased p53 Acetylation inhibits ferroptosis-induced MLE-12 cell death. An HS-treated MLE-12 cells model was established, in which cells were initially exposed to 42 °C conditions for 3 h and then incubated at 37 °C for 12 h. (A) Western blotting was used to determine p53 K382 acetylation and p53 protein expression of MLE-12 cells in the presence or absence of HS exposure; the representative Western blotting images were from three independent experiments. (B) MLE-12 cells were transfected with Ad-p53K382R for 2 days; fluorescence microscopy was used to validate Ad-p53K382R transfection; (C) Cells were transfected with Ad-p53K382R for 2 days before HS induction. All samples were collected at 12 h after HS induction; p53 K382 acetylation and p53 protein expression levels were determined by western blotting. (D) The LDH cytotoxicity was assayed at 12 h after HS induction. (E) Meanwhile, the MDA levels were assayed at 12 h following HS exposure. (F) SLC7A11, GPX4 and ACSL4 protein levels were determined by western blotting. Data are shown as the mean ± SD. *p < .05, **p < .01, #p < .05 in comparison with HS; n = 3.

Figure 6. The regulation of p53 acetylation expression by SIRT1 in MEL-2 cells. Cells were transfected with plasmid for 2 days before HS induction. All samples were collected at 12 h after heat stress. SIRT1, p53 K382 acetylation and p53 protein levels were determined by western blotting.
may play an important role in mediating lung epithelial ferroptosis in HS-ALI. This study may provide some new therapeutic strategies for targeting SIRT1 for future heat stroke treatment.

**Author contributions**

H.C., X.P.L., and X.H.Y. performed the study and composed this manuscript. X.F.L. and R.H.Y. were responsible for primary data generation and analysis. W.H.F. participated in cell culture and transfection. W.D.X. and Y.P.L. performed Western blots. W.D.X. and Y.N.L. was the principal investigator and corresponding author for these studies.

**Ethical approval**

All protocols of animal experiments were reviewed and approved by the Ethical Committee for Animal Experimentation of Nanfang Hospital.

**Disclosure statement**

No conflicts of interests are declared by the author(s).

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**References**

[1] Wang Y, Bobb JF, Papi B, et al. Heat stroke admissions during heat waves in 1,916 US counties for the period from 1999 to 2010 and their effect modifiers. Environ Health. 2016;15(1):83–92.

[2] Cheung AM, Tansey CM, Tomlinson G, et al. Two-year outcomes, health care use, and costs of survivors of acute respiratory distress syndrome. Am J Respir Crit Care Med. 2006;174(5):538–544.

[3] Zhou Q, Chen Z, Li J, et al. Role of the receptor for advanced glycation end products in heat Stress-Induced endothelial hyperpermeability in acute lung injury. Front Physiol. 2020;11:1087.

[4] Sawka MN, Leon LR, Montain SJ, et al. Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. Compr Physiol. 2011;1(4):1883–1928.

[5] Huang W, Xie W, Gong J, et al. Heat stress induces RIP1/RIP3-dependent necroptosis through the MAPK, NF-κB, and c-Jun signaling pathways in pulmonary vascular endothelial cells. Biochem Biophys Res Commun. 2020;528(1):206–212.

[6] Zhongyin Z, Wei W, Juan X, et al. Isoliquiritin apioside relieves intestinal ischemia/reperfusion-induced acute lung injury by blocking Hif-1α-mediated ferroptosis. Int Immunopharmacol. 2022;108:108852.

[7] He S, Li R, Peng Y, et al. ACSL4 contributes to ferroptosis-mediated rhabdomyolysis in exertional heat stroke. J Cachexia Sarcopenia Muscle. 2022;13(3):1717–1730.

[8] Lagan AD, Melley DD, Evans TW, et al. Pathogenesis of the systemic inflammatory syndrome and acute lung injury: role of iron mobilization and decompartmentalization. Am J Physiol Lung Cell Mol Physiol. 2008;294(2):L161–L174.

[9] Xia Z, Kon N, Gu AP, et al. Deciphering the acetylation code of p3 in transcription regulation and tumor suppression. Oncogene. 2022;41(22):3039–3050.

[10] Zhu W-G. Regulation of p3 acetylation. Sci China Life Sci. 2017;60(3):321–332.

[11] Li Y, Cao Y, Xiao J, Shang J, Tan Q, Ping F, Huang W, Wu F, Zhang H, Zhang X. Inhibitor of apoptosis-stimulating protein of p33 inhibits ferroptosis and alleviates intestinal ischemia/reperfusion-induced acute lung injury. Cell Death Differ. 2020;27(9):2635–2650.

[12] Jiang L, Kon N, Li T, et al. Ferroptosis as a p3-mediated activity during tumor suppression. Nature. 2015;520(7545):57–62.

[13] Li L, Su Z, Zou Z, et al. Ser46 phosphorylation of p35 is an essential event in prolyl-isomerase Pin1-mediated p35-independent apoptosis in response to heat stress. Cell Death Dis. 2019;10(2):1–16.

[14] Liu XM, Liu JY, Xiao W, et al. SIRT1 regulates N-6-methyladenosine RNA modification in hepatocarcinogenesis by inducing RANBP2-dependent FTO SUMOylation. Hepatology. 2020;72(6):2029–2050.

[15] Sun M, Li J, Mao L, et al. p33 deacetylation alleviates sepsis-induced acute kidney injury by promoting autophagy. Front Immunol. 2021;12:685523.

[16] Ma S, Sun L, Wu W, et al. USP22 protects against myocardial ischemia–reperfusion injury via the SIRT1-p33/SLC7A11-dependent inhibition of ferroptosis-induced cardiomyocyte death. Front Physiol. 2020;11:551318.

[17] Geng Y, Ma Q, Liu Y-N, et al. Heatstroke induces liver injury via IL-1β and HMGB1-induced pyroptosis. J Hepatol. 2015;63(3):622–633.

[18] Wang C, Liu T, Tong Y, et al. Ulcinastatin protects against acetaminophen-induced liver injury by alleviating ferroptosis via the SIRT1/NRF2/HO-1 pathway. Am J Transl Res. 2020;13(6):6031–6042.

[19] Brooks CL, Gu W. Ubiquitination, phosphorylation and acetylation: the event in prolyl-isomerase Pin1-mediated p35-independent apoptosis in response to heat stress. Cell Death Dis. 2019;10(2):1–16.

[20] Zhang W, Gai C, Ding D, et al. Targeted p33 on small-molecules-induced ferroptosis in cancers. Front Oncol. 2018;8:507.

[21] Wang C, Liu T, Tong Y, et al. Ulinastatin protects against acetaminophen-induced liver injury by alleviating ferroptosis via the SIRT1/NRF2/HO-1 pathway. Am J Transl Res. 2020;13(6):6031–6042.

[22] Zhang H, Zhang X. Inhibitor of apoptosis-stimulating protein of p33 mediates ferroptosis-dependent inhibition of ferroptosis–induced cardiomyocyte death. Front Physiol. 2020;11:551318.

[23] Sun M, Li J, Mao L, et al. p33 deacetylation alleviates sepsis-induced acute kidney injury by promoting autophagy. Front Immunol. 2021;12:685523.

[24] Ma S, Sun L, Wu W, et al. USP22 protects against myocardial ischemia–reperfusion injury via the SIRT1-p33/SLC7A11-dependent inhibition of ferroptosis–induced cardiomyocyte death. Front Physiol. 2020;11:551318.

[25] Geng Y, Ma Q, Liu Y-N, et al. Heatstroke induces liver injury via IL-1β and HMGB1-induced pyroptosis. J Hepatol. 2015;63(3):622–633.

[26] Wang C, Liu T, Tong Y, et al. Ulcinastatin protects against acetaminophen-induced liver injury by alleviating ferroptosis via the SIRT1/NRF2/HO-1 pathway. Am J Transl Res. 2020;13(6):6031–6042.

[27] Brooks CL, Gu W. Ubiquitination, phosphorylation and acetylation: the molecular basis for p33 regulation. Curr Opin Cell Biol. 2003;15(2):164–171.

[28] Zhang W, Gai C, Ding D, et al. Targeted p33 on small-molecules-induced ferroptosis in cancers. Front Oncol. 2018;8:507.

[29] Wang S-J, Li D, Ou Y, et al. Acetylation is crucial for p33-mediated ferroptosis and tumor suppression. Cell Rep. 2016;17(2):366–373.

[30] Li J, Cao F, Yin H-L, et al. Ferroptosis: past, present and future. Cell Death Dis. 2020;11(2):88.

[31] Doll S, Proneth B, Tuylina Y, et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. Nat Chem Biol. 2017;13(1):91–98.

[32] Chen C, Zhou M, Ge Y, et al. SIRT1 and aging related signaling pathways. Mech Ageing Dev. 2015;136(2):172–173.

[33] Nagpal I, Yuan Z-M. The basally expressed p53-Mediated homeostatic function. Front Cell Dev Biol. 2021;9:775312.

[34] Vainer R, Cohen S, Shahar A, et al. Structural basis for p33 Lys120-acetylation-dependent DNA-binding mode. J Mol Biol. 2016;428(15):3013–3025.
[27] Wang Y, Chen Y, Chen Q, et al. The role of acetylation sites in the regulation of p53 activity. Mol Biol Rep. 2020;47(1):381–391.
[28] Alhebshi H, Tian K, Patnaik L, et al. Evaluation of the role of p53 tumour suppressor posttranslational modifications and TTC5 cofactor in lung cancer. Int J Mol Sci. 2021;22(24):13198.
[29] Xu S, Jiang B, Hou X, et al. High-fat diet increases and the polyphenol, S17834, decreases acetylation of the sirtuin-1-dependent lysine-382 on p53 and apoptotic signaling in atherosclerotic lesion-prone aortic endothelium of normal mice. J Cardiovasc Pharmacol. 2011;58(3):263–271.
[30] Chandel N, Ayasolla K, Wen H, et al. Vitamin D receptor deficit induces activation of renin angiotensin system via SIRT1 modulation in podocytes. Exp Mol Pathol. 2017;102(1):97–105.