论著·基础医学

# 微小RNA-21-5p通过STAT3/P53/SLC7A11轴抑制铁死亡减轻高氧性急性肺损伤

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[摘要] **目的** 探讨微小miR-21-5p(microRNA-21-5p,miR-21)是否通过STAT3/P53/SLC7A11轴调控铁死亡在高氧性急性肺损伤(hyperoxia-induced acute lung injury,HALI)发挥肺保护作用。**方法** 采用免疫共沉淀技术(co-immunoprecipitation, Co-IP)分析STAT3与P53的相互作用。选择50只9周龄C57BL/6J雄性小鼠,按随机数字表法分为常氧(Control)组、高氧(HALI)组、miR-21过表达(miR-21)组、STAT3抑制剂(HY-13818)组及铁抑素-1(ferrostatin-1,Fer-1)组。Control组置于室内空气中饲养;miR-21组经气管导管滴入miR-21 AAV6载体,饲养3周;HY-13818组以10 mg/kg经腹腔注射,每周3次,干预2周;Fer-1组在造模时以0.8 mg/kg ferrostatin-1经尾静脉注射,每天1次,干预2次;吸入90%以上浓度氧气建立HALI小鼠模型。高氧暴露48 h后,眼球采血RT-PCR检测miR-21表达水平;取肺组织计算肺$W_{湿质量}/D_{干质量}$,HE染色光镜下观察形态学变化并行肺损伤病理评分;光电比色法测定超氧化物歧化酶(superoxide dismutase,SOD)水平,可见分光光度法测定丙二醛(malondialdehyde,MDA)、$Fe^{2+}$及谷胱甘肽(glutathione,GSH)浓度,荧光分光光度法测定活性氧(ROS)水平;Western blot法检测肺组织STAT3、P53、SLC7A11及GPX4蛋白相对表达水平。**结果** Co-IP结果显示,STAT3可与P53结合。HALI组HE染色可见肺泡结构破坏、排列紊乱、间隔明显增厚伴大量中性粒细胞细胞浸润,肺泡萎陷,与Control组比较,肺损伤病理评分及肺$W_{湿质量}/D_{干质量}$显著增高($P<0.05$);HE染色结果显示,miR-21组、HY-13818组及Fer-1组肺损伤程度较HALI明显减轻,肺损伤病理评分及$W_{湿质量}/D_{干质量}$比显著降低($P<0.05$)。与Control组比较,HALI组MDA、$Fe^{2+}$及ROS水平升高($P<0.05$),SOD、GSH水平降低($P<0.05$),STAT3及P53蛋白表达升高($P<0.05$),SLC7A11及GPX4蛋白表达下降($P<0.05$);与HALI组比较,miR-21组和HY-13818组MDA、ROS水平降低($P<0.05$),SOD、$Fe^{2+}$及GSH水平升高($P<0.05$),STAT3、P53表达降低($P<0.05$),SLC7A11及GPX4蛋白升高($P<0.05$)。**结论** miR-21减轻高氧性急性肺损伤,可能与其通过STAT3/P53/SLC7A11轴抑制铁死亡相关。

[关键词] 微小RNA-21-5p;铁死亡;STAT3/P53/SLC7A11信号通路;高氧性急性肺损伤

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# MiR-21-5p alleviates hyperoxia-induced acute lung injury by inhibiting ferroptosis through the STAT3/P53/SLC7A11 axis

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[**Abstract**] Objective To investigate whether microRNA-21-5p (miR-21) plays a protective role in hyperoxia-induced acute lung injury (HALI) by regulating ferroptosis through the STAT3/P53/SLC7A11 axis. Methods The interaction between STAT3 and P53 was analyzed by co-immunoprecipitation (Co-IP). Fifty 9-week-old male C57BL/6J mice were randomly divided into normoxia (Control) group, HALI group, miR-21 overexpression (miR-21) group, STAT3 inhibitor (HY-13818) group, and ferrostatin-1 (Fer-1) group. After the mice from the miR-21 group received miR-21-5p AAV6 or empty vector *via* tracheal catheter instillation, the animals were then monitored for 3 weeks. The HY-13818 group was intraperitoneally injected with HY-13818 (10 mg/kg) 3 times weekly for 2 weeks. The Fer-1 group received 0.8 mg/kg ferrostatin-1 *via* tail vein injection once daily for 2 consecutive days during modeling. The HALI model was established by exposure to >90% oxygen. After 48 h of hyperoxia, blood samples were collected *via* orbital sampling for RT-PCR analysis of miR-21 expression. Lung tissues were harvested for wet/dry weight ratio and assessment of histopathological changes *via* HE staining for lung injury score. Activity of superoxide dismutase (SOD) and contents of malondialdehyde (MDA), $Fe^{2+}$, glutathione (GSH), and reactive oxygen species (ROS) were measured using photocolorimetry, spectrophotometry and fluorometry. Western blotting was used to evaluate the protein expression of STAT3, P53, SLC7A11, and GPX4. Results The results of Co-IP showed that STAT3 could bind to P53. The HALI group exhibited obviously destroyed alveolar structure, disordered arrangement, thickened interval, with a large number of infiltrated neutrophils and collapsed alveoli, and had significantly increased pathological score of lung injury and ratio of lung $W_{wet}/D_{dry}$ weight when compared with the Control group ($P<0.05$). In the miR-21 group, HY-13818 group and Fer-1 group, the severity of lung injury was significantly reduced, and the pathological score of lung injury and the ratio of $W_{wet}/D_{dry}$ weight were decreased ($P<0.05$) when compared with the HALI group. Compared with the control group, the contents of MDA, $Fe^{2+}$ and ROS were increased ($P<0.05$), the activity of SOD and content of GSH were declined ($P<0.05$), the protein levels of STAT3 and P53 were increased ($P<0.05$), and those of SLC7A11 and GPX4 were decreased ($P<0.05$) in the HALI group. Compared with the HALI group, decreased MDA and ROS levels ($P<0.05$), enhanced SOD activity, $Fe^{2+}$ and GSH levels ($P<0.05$), down-regulation of STAT3 and P53 ($P<0.05$) and up-regulation of SLC7A11 and GPX4 ($P<0.05$) were observed in the miR-21 group and HY-13818 group. Conclusion MiR-21 alleviates HALI, which may be related to its inhibition of ferroptosis through the STAT3/P53/SLC7A11 axis.

[**Key words**] microRNA-21-5p; ferroptosis; STAT3/P53/SLC7A11 signaling pathway; hyperoxic acute lung injury

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严重低氧血症的危重患者常常需要给予超生理浓度氧气以改善氧合,但长时间高氧性机械通气的氧分压大于300 mmHg时常导致高氧性急性肺损伤(hyperoxia-induced acute lung injury,HALI)的发生,其不但促进急性呼吸窘迫综合征(acute respiratory distress syndrome,ARDS)的进展,甚至增加脓毒症患

者病死率,与重症监护病房(ICU)危重患者死亡风险密切相关[1]。HALI发病机制主要涉及炎症级联反应、凋亡/坏死、氧化应激及自噬等学说,至今尚未阐明[2]。铁死亡是以铁超载、脂质过氧化蓄积与胱氨酸/谷氨酸反向转运体(cystine/glutamate antiporter system,system Xc-)功能衰竭为特征的一种新型程序性细胞死亡方式[3-4]。目前研究认为,二价铁离子大量蓄积介导的线粒体功能障碍、脂质过氧化及氧化还原系统失衡在HALI的发生发展中起关键作用,靶向铁死亡可能是治疗HALI的有效治疗策略[3,5]。信号转导和转录激活因子3(signal transducer and activator of transcription 3,STAT3)作为STATs家族成员的核心成员之一参与了铁死亡的进展,研究显示,其过度活化可促进铁死亡加重心脏损伤[6],抑制其过度转录激活可减轻脓毒症急性肺损伤[7],其调控铁死亡的机制尚在探索阶段,值得进一步研究。

微小RNA(microRNA,miRNA)是一类长度在21~24个核苷酸的非编码单链RNA,调控了大约30%的蛋白质编码基因,在细胞分化/增殖、氧化应激、凋亡/坏死、自噬等病理生理过程中起关键作用。现有研究表明,miRNA在调控铁死亡的机制中不可或缺[8],被认为是靶向治疗ARDS的重要途径[9]。其中,miR-21被认为是治疗ARDS的核心靶点及预后标记物[10]。课题组前期研究表明,miR-21可靶向STAT3抑制肺泡细胞凋亡[11],在防治HALI中起核心作用[11-12],但其调控铁死亡在HALI中的作用机制,国内外鲜见报道。本研究拟通过构建HALI小鼠模型验证miR-21通过STAT3调控铁死亡在HALI中的可能机制,以期阐明miR-21、STAT3与铁死亡在HALI中的作用,确立其为治疗HALI的核心靶点。

## 1 材料与方法

### 1.1 实验动物

50只8周龄C57BL/6J雄性小鼠,体质量(22±2)g,购自天勤生物科技公司(长沙),动物合格证号:SCXK(湘)2021-0004。动物实验获得遵义市中医院伦理委员会批准[伦审(2023)HYSC20号],实验动物处置方法遵循动物伦理学3R准则。各组小鼠分笼饲养于遵义医科大学动物实验中心:温度(22±2)℃,相对湿度(60±10)%,昼夜循环照明(12 h/12 h)交替的环境下适应性喂养1周后进行实验。

### 1.2 主要试剂及仪器

小鼠肺上皮细胞MLE-12细胞系购于上海富衡生物科技有限公司;超氧化物歧化酶(duperoxide dismutase,SOD)、丙二醛(malondialdehyde,MDA)试剂盒、亚铁离子($Fe^{2+}$)与还原型谷胱甘肽(glutathione,GSH)含量检测试剂盒及BCA蛋白定量试剂盒购自北京索莱宝公司;STAT3、肿瘤蛋白53(tumor protein 53,P53)、谷胱甘肽过氧化物酶4(glutathione peroxidase 4,GPX4)、SLC7A11和β-actin一抗及辣根过氧化物酶标记的二抗购于美国CST公司;DCFH-DA活性氧(ROS)荧光探针、HE染色试剂盒购自碧云天生物公司;STAT3抑制剂HY-13818及铁抑素-1(ferrostatin-1,Fer-1)购于美国MCE生物科技公司(上海);miRNA第一链cDNA合成试剂盒、miRNA荧光定量PCR试剂盒及miR-21和U6 PCR引物设计合成(上海生工生物);miR-21腺相关病毒-6(AAV-6)过表达载体(汉恒生物)。酶标仪和电泳仪购自美国Bio-Rad公司;光学显微镜购自日本奥林巴斯(Olympus)公司。

### 1.3 实验动物分组、模型制备及给药方法

50只C57BL/6J按随机数表法分为常氧(Control)组、高氧(HALI)组、miR-21过表达(miR-21)组、STAT3抑制剂(HY-13818)组及铁抑素-1(ferrostatin-1,Fer-1)组。Control组饲养于正常空气中;miR-21组经气管导管滴入50 μL滴度为($1\times10^{12}$ TU/mL)的miR-21 AAV6过表达载体,饲养3周;HY-13818组HY-13818以10 mg/kg经腹腔注射,每周3次,干预2周;Fer-1组在造模时ferrostatin-1以0.8 mg/kg经尾静脉注射,每天1次,干预2次。除Control组外,其余4组参照刘国跃等[13]构建大鼠HALI动物模型:将实验用C57BL/6J小鼠饲养于55 cm×40 cm×35 cm的密闭有机玻璃容器自制高氧箱中,右侧开孔接单向阀控制的氧气接头;左侧开孔接氧浓度和$CO_2$浓度检测仪。箱底放置钠石灰吸收$CO_2$,维持其$CO_2$浓度<0.5%;每天持续给氧23.5 h,维持氧浓度90%以上,建模48 h,2%戊巴比妥纳以4.5 mg/100 g腹腔注射麻醉小鼠后,眼球摘除法采血1.5 mL后处死小鼠取肺组织进行下一步实验。

### 1.4 免疫共沉淀(co-immunoprecipitation,Co-IP)分析STAT3与P53相互作用

取培养好的MLE-12细胞,加入适量高效RIPA裂解液冰上裂解20 min,4 ℃离心10 min(2 000 r/min)后取上清;1 μg IgG抗体和P53抗体加入到细胞裂解液,4 ℃孵育过夜形成抗原-抗体复合物;取10 μL proteinA/G磁珠PBS冲洗后离心5 min(3 000 r/min);将其加入到孵育抗体过夜的细胞裂解液中,4 ℃孵育3 h(摇床)后4 ℃离心5 min(3 000 r/min),见proteinA/G磁珠沉淀;弃上清液,proteinA/G磁珠冲洗后加入6 μL的5×SDS上样缓冲液,沸水煮8 min;进行SDS-PAGE电泳,

Western blot分析STAT3与P53的相互作用。

1.5 光学显微镜观察各组小鼠肺组织病理学改变并进行肺损伤病理评分

取小鼠左肺上叶肺组织,按照HE染色标准操作步骤,4%多聚甲醛固定24 h,梯度脱水,石蜡包埋后切片(厚度5 μm),HE染色后脱水封片,光学显微镜下观察肺组织损伤改变;每组大鼠($n$=6)HE染色切片随机选取至少20个高倍视野(×400),观察肺泡中中性粒细胞个数(A)、肺泡间质中性粒细胞个数(B)、透明膜个数(C)、被蛋白质碎片填充的肺泡个数(D)及肺泡间隔厚度(E),采用MATUTE-BELLO等[14]肺损伤评分系统进行肺损伤标准化评分。

1.6 各组小鼠肺组织肺$W_{湿质量}/D_{干质量}$比

取小鼠左肺下叶肺组织滤纸吸干水分称重,记为肺组织湿质量(W),放入60 ℃烤箱烘烤72 h后称重,记为肺组织干质量(D),按如下公式计算:肺湿质量/干质量比=肺$W_{湿质量}$/肺$D_{干质量}$。

1.7 各组小鼠肺组织SOD、$Fe^{2+}$、GSH、MDA及ROS水平测定

取肺组织称重后剪碎研磨成匀浆,1 000×$g$离心10 min,吸取上清,操作严格按试剂盒说明书进行,采用光电比色法测定SOD水平,可见分光光度法测定MDA、$Fe^{2+}$及GSH浓度,取肺组织剪碎胰酶消化后过滤制成单细胞悬液(细胞数不少于$10^6$个),加入DCFH-DA荧光探针,荧光分光光度法测定ROS水平。

1.8 小鼠血清miR-21表达检测

使用血清标本,加入Trizol裂解液提取RNA,酶标仪测定总RNA浓度及纯度。使用miRNA第一链cDNA合成试剂盒(加尾法)将总RNA逆转录为cDNA,进行定量PCR扩增。采用SYBR Green Ⅰ染料法对microRNA进行荧光定量检测:反应体系为20 μL:2×miRNA qPCR master mix 10 μL;前后引物各1 μL;Template DNA(miR-21)2 μL;ROX Reference Dye(L)/(H),1 μL;加入RNase-free water至20 μL。PCR反应条件为:预变性95 ℃、30 s;变性95 ℃、5 s;退火/延伸60 ℃、30 s;共40个循环。引物序列为:miR-21正向5′-CCGGCTAGCTTATCAGACTGATGTTGA-3′,反向5′-CTCAACTGGTGTCGTGGA-3′;U6正向5′-CTCGCTTCGGCAGCACA-3′,反向5′-AACGCTTCACGAATTTGCGT-3';采用相对定量法检测RNA扩增,以U6作内参,采用$2^{-\Delta\Delta Ct}$法计算miR-21表达量。

1.9 Western blot法检测各组小鼠肺组织STAT3、P53、GPX4、SLC7A11蛋白表达水平

取各组小鼠肺组织称重80 mg,加入RIPA裂解液、蛋白酶和磷酸酶抑制剂裂解组织后,4 ℃ 12 000×$g$离心30 min,取上清液使用BCA法进行蛋白定量并调整至一致浓度,样品经10%或12%SDS-PAGE电泳分离,转膜后于5%脱脂奶粉封闭2 h,分别加入STAT3(1∶2 000)、P53(1∶2 000)、GPX4(1∶2 000)、SLC7A11(1∶2 000)及β-actin(1∶4 000)抗体4 ℃过夜,洗膜后加入IgG-HRP二抗(1∶2 000)后室温避光孵育1 h,洗膜后加入ECL发光液,使用Gel Doc™ EZ全自动凝胶成像系统曝光、拍照。Image J软件进行条带灰度值分析。以β-actin为内参照,计算目标蛋白相对表达量=目的蛋白条带灰度值/内参条带灰度值。

1.10 统计学分析

数据分析采用IBM SPSS 29.0统计软件,符合正态分布的计量资料以$\bar{x}\pm s$表示,多组比较采用单因素方差分析,两两比较采用LSD-$t$检验,$P$<0.05为差异具有统计学意义。

## 2 结果

2.1 免疫共沉淀结果显示STAT3蛋白可结合P53蛋白

通过转染P53抗体检测STAT3与P53是否存在物理结合。在MLE-12细胞中,用P53进行免疫共沉淀实验,Western blot检测STAT3和P53的表达,发现STAT3蛋白可结合P53蛋白(图1)。

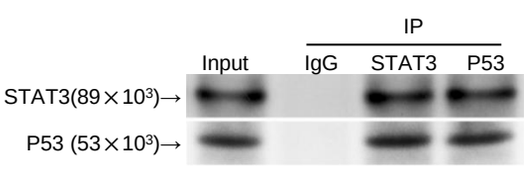



**图1 STAT3与P53的蛋白质免疫共沉淀结果**

**Figure 1 Results of co-immunoprecipitation (Co-IP) between STAT3 and P53**

2.2 各组小鼠肺组织形态学、$W_{湿质量}/D_{干质量}$、肺损伤病理评分及血清miR-21变化

Control组HE染色见肺泡排列整齐,肺泡结构清晰,肺泡壁光滑;肺泡间隔偶有3~5个中性粒细胞浸润;HALI组肺泡排列结构紊乱、肺泡间隔明显增厚伴大量中性粒细胞浸润,可见大量萎陷及通明膜形成;miR-21组、HY-13818组及Fer-1组肺泡结构基本完整,肺泡间隔无显著增厚,中性粒细胞浸润显著减少(图2)。与Control组比较,HALI组肺损伤病理评分升高($P$<0.05);肺$W_{湿质量}/D_{干质量}$比值升高($P$<0.05);与HALI组比较,miR-21组、HY-13818组及Fer-1组肺损伤病理

评分降低($P$<0.05),肺 $W_{湿质量}/D_{干质量}$ 比下降($P$<0.05,图3A、B)。与Control组比较,HALI组miR-21表达水平降低($P$<0.05);与HALI组比较,miR-21组表达水平显著升高($P$<0.05,图3C)。

2.3 各组小鼠肺组织SOD、MDA、$Fe^{2+}$、GSH及ROS比较

与Control组比较,HALI组SOD、GSH明显降低($P$<0.05),MDA、$Fe^{2+}$及ROS明显升高($P$<0.05);与HALI组比较,miR-21组、HY-13818组及Fer-1组SOD、GSH明显升高($P$<0.05);MDA、$Fe^{2+}$及ROS明显降低($P$<0.05,图4)。

2.4 miR-21对HALI小鼠肺组织STAT3、P53、SLC7A11与GPX4蛋白表达的影响

与Control组比较,HALI组STAT3、P53蛋白表达升高($P$<0.05),SLC7A11、GPX4蛋白表达降低($P$<0.05,图5A)。与HALI组比较,miR-21组与HY-13818

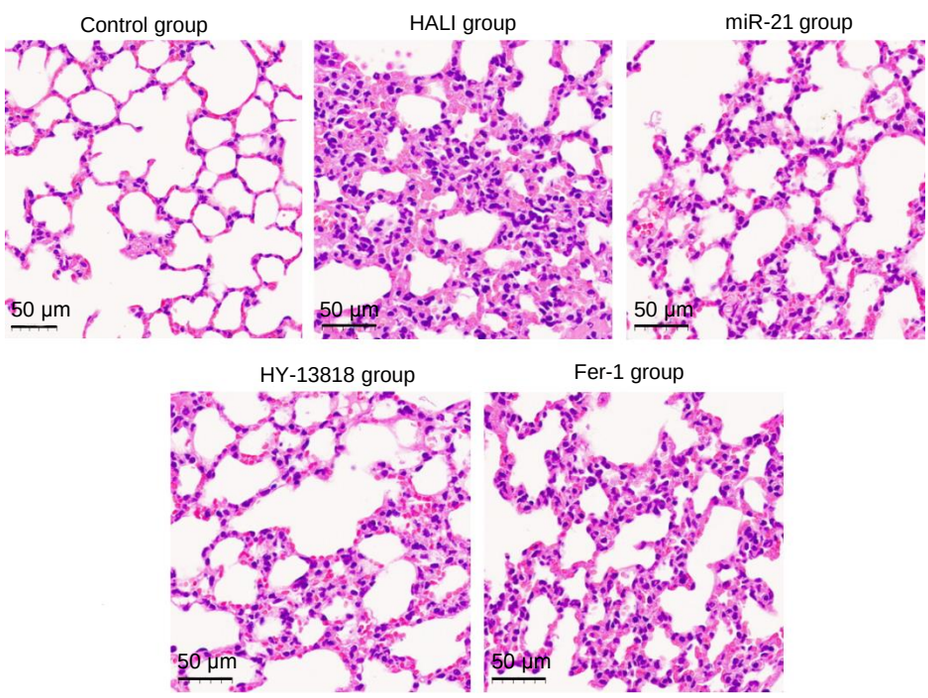
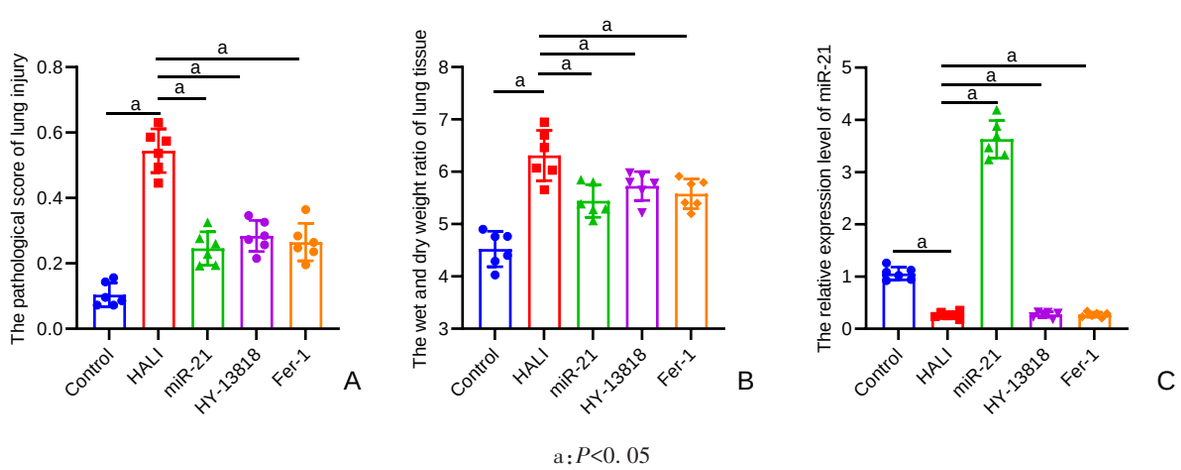



图2 各组小鼠肺组织HE染色结果

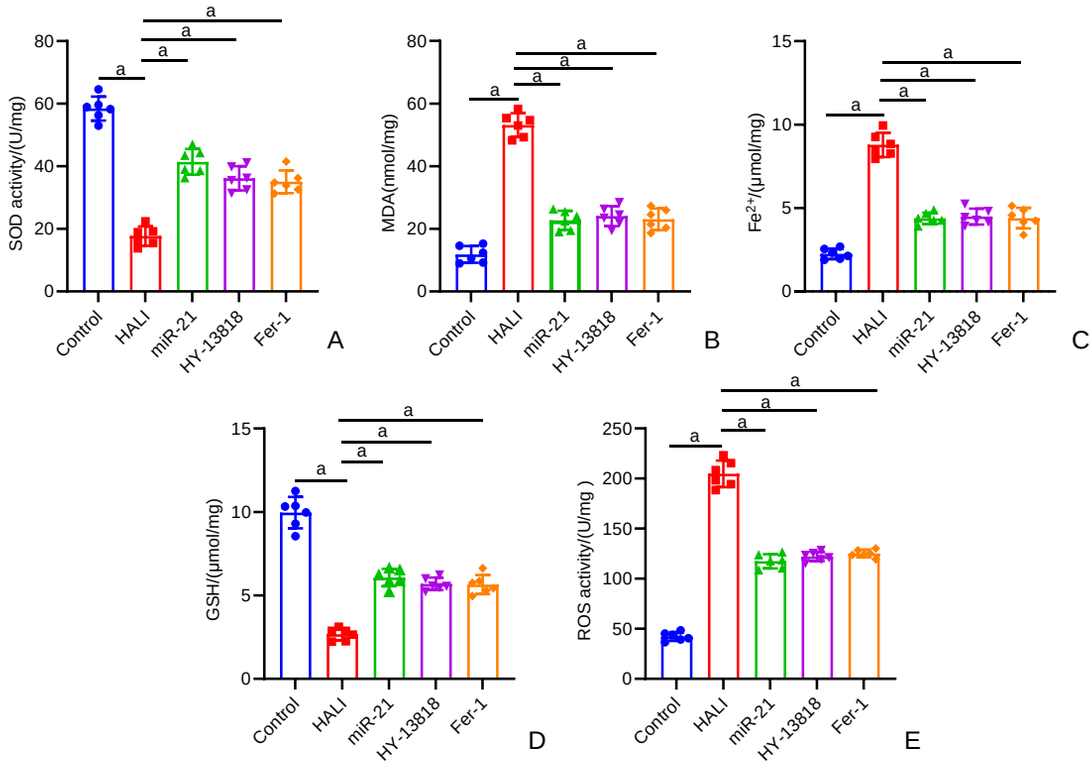
**Figure 2 HE staining showing lung pathology of mice in each group**




a:$P$<0.05

图3 各组小鼠肺损伤病理评分(A)、肺 $W_{湿质量}/D_{干质量}$(B)及血清miR-21表达水平(C)比较($n$=6)

**Figure 3 Comparison of lung injury pathology score (A), wet/dry weight ratio (B) and serum miR-21 expression (C) in mice of each group ($n$=6)**




a:$P$<0.05

**图4 各组小鼠肺组织SOD(A)、MDA(B)、$Fe^{2+}$(C)、GSH(D)及ROS(E)比较**($n$=6)

**Figure 4 Comparison of SOD (A), MDA (B), $Fe^{2+}$(C), GSH (D) and ROS(E) in lung tissue of mice in each group**($n$=6)

组STAT3、P53蛋白表达降低($P$<0.05,图5B、C);SLC7A11、GPX4蛋白表达升高($P$<0.05,图5D、E)。

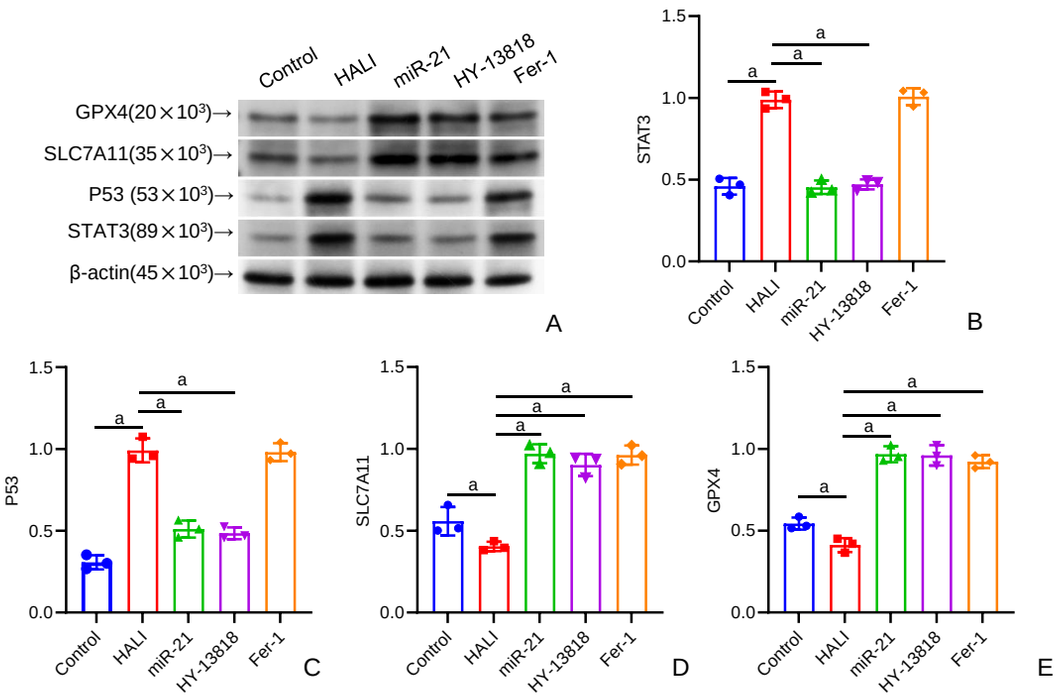
## 3 讨论

### 3.1 miR-21干预减轻高氧性急性肺损伤

目前研究认为,高氧暴露可抑制线粒体电子传递链减少ATP产生,造成线粒体ROS蓄积加重线粒体DNA损伤[15],进一步促进ROS的产生,ROS不仅促进炎症级联反应,还促进$Fe^{2+}$蓄积,被认为是HALI的始动因素[5]。而$Fe^{2+}$介导的氧化应激损伤被认为是HALI的重要标志,故铁死亡被确定为HALI的核心致病机制之一[5]。本研究通过HE染色发现HALI组肺泡结构严重破坏,肺间隔明显增厚伴中性粒细胞大量浸润,大量肺泡萎陷,同时肺损伤病理及肺组织$W_{湿质量}/D_{干质量}$比显著升高,提示高氧暴露可导致肺水肿和炎症反应加剧。而miR-21组、HY-13818组及Fer-1组的肺泡结构完整性明显恢复,肺水肿和炎症浸润显著减轻,且3组间改善效果无显著差异,表明miR-21抑制铁死亡发挥肺保护作用可能与STAT3活性抑制及铁死亡抑制相关。此外,miR-21的过表达减轻了HALI并抑制了铁死亡,提示miR-21可能是缓解肺损伤的关键分子。这一结果与既往研究一致,即miR-21在急性肺损伤中具有抗炎和抗细胞死亡的作用[16-17],但其通过调控铁死亡的机制尚未明确,本实验为此提供了新证据。

### 3.2 高氧性急性肺损伤涉及脂质过氧化介导的铁死亡

铁死亡的主要特征是$Fe^{2+}$介导的芬顿反应造成大量蓄积的ROS直接与多不饱和脂肪酸(polyunsaturated fatty acid,PUFA)结合导致脂质过氧化物(lipid peroxide,LPO)大量蓄积;以及System Xc-障碍引起的GPX4失活[3-4]。研究显示,GSH作为体内的核心抗氧化剂之一主要受System Xc-调控,其不仅可以清除$H_2O_2$、$\cdot O_2^-$等自由基,在细胞的氧化应激中发挥核心作用,还作为GPX4的辅因子以维持GPX4的活性可将LPO纯化,发挥抗铁死亡作用[18]。SOD与MDA作用评估氧化应激水平的核心指标,常用于各类实验的氧化应激评估。本研究中,HALI组MDA、$Fe^{2+}$、ROS升高,SOD、GSH降低,而经miR-21过表达后,MDA、$Fe^{2+}$、ROS降低,SOD、GSH降低,与HY-13818组结果




A: Western blotting results of protein expression in lung tissues of each group; B~E: Relative expression levels of STAT3 (B), P53 (C), SLC7A11 (D) and GPX4(E). a: $P<0.05$

**图5 各组小鼠STAT3、P53、SLC7A11、GPX4蛋白相对表达水平（$n$=3）**

**Figure 5 Relative protein levels of STAT3, P53, SLC7A11, and GPX4 in lung tissue of mice in each group（$n$=3）**

相似，表明miR-21过表达通过调控氧化应激减轻HALI，其机制可能与STAT3密切相关，这与之前的研究一致[11]。此外，使用铁死亡抑制剂后，HALI肺损伤明显缓解，表明铁死亡这一病理过程促进了HALI的进展，与$Fe^{2+}$及ROS蓄积不能被有效清除密切相关，其机制可能与GSH表达抑制和GPX4失活密切相关[19]。基于转录组学的研究结果表明，HALI通过多种途径及机制抑制GSH与GPX4活性[19]。此外，研究显示，在HALI的新生小鼠模型中，高氧显著降低GSH和GPX4水平，同时增加脂质过氧化主要产物MDA水平[20]，本实验与其结果一致。

### 3.3 抑制STAT3/P53/SLC7A11轴可调控铁死亡

既往研究显示，STAT3过度激活可通过氧化应激失衡、细胞焦亡及凋亡等机制促进ARDS进展，而抑制STAT3表达可减轻脓毒症急性肺损伤[7,21]。最近的研究认为，STAT3与铁死亡密切相关，抑制STAT3可通过调控铁死亡减轻脓毒症急性肺损伤[22-23]。其机制可能涉及P53转录，研究显示，STAT3激活可促进P53表达，导致GPX4失活促进铁死亡[24]，然而STAT3与P53的调控关系错综复杂，文献报道不一。课题组前期研究发现[19]，P53在HALI中高表达与铁死亡密切相关，ROS通过激活STAT3抑制miR-21表达促进肺泡Ⅱ型细胞凋亡加重HALI[16]。而过表达miR-21可靶向STAT3减轻HALI[11]。本实验通过免疫共沉淀技术发现STAT3可与P53结合，使用STAT3抑制剂后P53表达明显降低，表明STAT3正向调控P53表达，这与HUANG等[25]及PROSDOCIMI等[26]的研究一致。而P53可负向调控SLC7A11转录促进铁死亡[27]。本研究中，抑制STAT3表达后，P53表达降低，与使用铁死亡抑制剂(ferrostatin-1)效果相似，SLC7A11及GPX4表达升高，表明miR-21可经STAT3-P53/ SLC7A11调控铁死亡减轻HALI。

### 3.4 miR-21抑制铁死亡可能与抑制STAT3/P53/SLC7A11轴密切相关

miRNA是一类进化上高度保守、长度约21～24个核苷酸的非编码单链RNA，其通过与靶基因3′非编码区(3′-UTR)结合，在转录后水平调控约30%的蛋白质编码基因表达。其通过调控氧化还原平衡、细胞分化/增殖、凋亡/坏死、自噬及炎症反应等病理生理过程，在肺部疾病的发生发展中起关键作用。研究表明，miRNA不仅参与铁死亡调控机制，还被视作ARDS的潜在治疗靶点[8-9]。其中，miR-21被确认为核心调控分子，兼具治疗靶点、生物标志物和预后评估指标的多重价值[10]。课题组前期研究发现，miR-21可通过靶

向STAT3抑制肺泡细胞凋亡,在防治HALI中发挥核心作用[11,17]。结合既往研究及本实验结果,STAT3作为miR-21的潜在下游靶点[11],可能通过激活P53进一步抑制SLC7A11的表达,从而加剧氧化应激和铁死亡。而miR-21的上调可能通过抑制STAT3活性,解除P53对SLC7A11的转录抑制,促进GSH合成并增强抗氧化能力。这一调控轴在HY-13818组中同样被模拟,表明STAT3/P53/SLC7A11通路可能是miR-21抗铁死亡的核心途径。此外,Fer-1组与miR-21组的表型高度相似,进一步验证铁死亡在HALI中的关键作用,以及miR-21干预的机制特异性。

本研究揭示了miR-21通过STAT3/P53/SLC7A11轴抑制铁死亡以减轻HALI的分子机制,为高氧性肺损伤提供了潜在治疗靶点。miR-21的过表达不仅缓解了病理损伤,还显著恢复了氧化/抗氧化系统的平衡。然而,本研究仍存在局限性:①本实验仅在动物实验中证实,尚待细胞实验进一步验证;②本研究未进行STAT3过表达挽救实验以观察过表达STAT3是否逆转miR-21对P53、SLC7A11的调控效应,课题组将在后续研究中进一步完善相关实验探索miR-21调控STAT3/P53/SLC7A11轴的分子机制;③需对P53基因进行过表达及沉默并通过Co-IP或荧光共振能量转移实验以强调STAT3-P53复合物对SLC7A11的调控作用。未来研究可结合基因敲除动物模型和临床样本,深入探索该通路的转化医学价值。

**利益冲突** 本文所有作者声明不存在利益冲突

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