论著·基础医学

富马酸二甲酯通过激活NRF2/SLC7A11轴抑制肺上皮细胞铁死亡并调控巨噬细胞极化缓解高原低氧急性肺损伤

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［摘要］ **目的** 探讨核因子红细胞系2相关因子2(nuclear factor erythroid 2-related factor 2,NRF2)激动剂富马酸二甲酯(dimethyl fumarate,DMF)对高原低氧急性肺损伤的保护作用,为开发高原低氧急性肺损伤预防新策略奠定基础。**方法** ①SPF级5~6周龄雄性Wistar大鼠20只,体质量210~230 g,按随机数字表法分为(n=5):常氧对照组(连续10 d腹腔注射生理盐水后置于常压常氧喂养)、低氧肺损伤组(连续10 d腹腔注射生理盐水后置于模拟海拔5 000 m的低压氧舱中暴露48 h)、DMF对照组(连续10 d腹腔注射DMF后置于常压常氧环境正常喂养)和DMF预防组(连续10 d腹腔注射DMF后置于低压氧舱中暴露48 h)。随后取大鼠肺组织用HE染色观察各组形态学变化并行肺损伤病理评分,ELISA检测炎症因子检测观察各组炎症反应,免疫荧光法检测肺组织活性氧(reactive oxygen species,ROS)水平,可见分光光度法测定谷胱甘肽(glutathione,GSH)、丙二醛(malondialdehyde, MDA)含量以及超氧化物歧化酶(super oxide dismutase,SOD)活性,Western blot和RT-qPCR检测铁死亡通路蛋白的水平变化情况。②将人支气管上皮(human bronchial epithelial, BEAS-2B)细胞和由人类外周血单核细胞(tumor human peripheral blood monocytes-1,THP-1)诱导的巨噬细胞置于常氧条件和低氧条件下共培养48 h,建立常氧对照组细胞模型和低氧肺损伤细胞模型,采用液相色谱-串联质谱法(liquid chromatography-tandem mass spectrometry,LC-MS/MS)比较常氧对照组和低氧肺损伤组BEAS-2B细胞的蛋白变化情况,并用生物信息学分析关键差异蛋白。BEAS-2B细胞预先DMF给药24 h后与THP-1细胞共培养作为DMF预防组,检测BEAS-2B细胞铁死亡相关指标及巨噬细胞极化及炎症因子分泌相关指标。在DMF干预的基础上敲低和过表达溶质载体家族7成员11(solute carrier family 7 member 11,SLC7A11)基因,检测BEAS-2B细胞的铁死亡相关指标及巨噬细胞极化和炎症因子分泌相关指标。**结果** ①与低氧肺损伤组相比,DMF预防组肺组织ROS水平和MDA含量降低(P<0.05),SOD活性和GSH含量升高(P<0.05),SLC7A11、GSH过氧化物酶4(glutathione peroxidase 4, GPX4)表达升高(P<0.05),同时肺损伤评分及炎症因子表达水平显著降低(P<0.05)。②BEAS-2B细胞蛋白组学共检测出5 377个蛋白,筛选到613个差异表达蛋白(differentially expressed proteins,DEPs),包括302个上调蛋白和311个下调蛋白,9个与铁死亡相关的差异表达蛋白中SLC7A11变化最为明显。在高原低氧肺损伤的细胞模型中,与低氧肺损伤组相比,DMF预防组BEAS-2B细胞的SLC7A11、GPX4表达升高(P<0.05),GSH水平及SOD活性升高,巨噬细胞M1型比例降低且炎症因子水平下降(P<0.05)。③在DMF预防组基础上敲减SLC7A11后,逆转了DMF对BEAS-2B细胞MDA含量的降低(P<0.05),以及SOD活性和GSH含量的升高(P<0.05),且敲减SLC7A11后GPX4表达降低(P<0.05),巨噬细胞M1型比例有所升高,分泌的炎症因子水平显著升

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高(P<0.05)。相反,过表达SLC7A11进一步增强了DMF的作用,DMF预防过表达组相较于DMF预防组BEAS-2B细胞GSH水平、SOD活性和GPX4表达显著升高(P<0.05),而MDA含量显著降低(P<0.05);同时过表达SLC7A11进一步缓解了炎症反应,IL-1、IL-6、TNF-α表达水平显著降低(P<0.05)。**结论** DMF通过上调NRF2/SLC7A11轴改善低氧ALI肺上皮细胞铁死亡,调控巨噬细胞极化状态并减轻炎症反应,从而对高原低氧ALI发挥保护作用。

[**关键词**] 高原低氧;肺损伤;铁死亡;炎症反应;巨噬细胞;NRF2/SLC7A11
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Dimethyl fumarate alleviates high-altitude hypoxic acute lung injury by activating the NRF2/SLC7A11 axis to inhibit pulmonary epithelial ferroptosis and regulate macrophage polarization

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[**Abstract**] **Objective** To investigate the protective effects of dimethyl fumarate (DMF), an agonist of nuclear factor erythroid 2-related factor 2 (NRF2), against acute lung injury (ALI) induced by high-altitude hypoxia, in order to found a basis for developing novel preventive strategies against hypoxic pulmonary injury. **Methods** ① Twenty SPF-grade male Wistar rats (5 to 6 weeks old, 210 to 230 g) were randomly divided into (n=5): normoxic control (intraperitoneal injection of normal saline for 10 consecutive days followed by being placed in a normobaric normoxia chamber), hypoxic lung injury (intraperitoneal injection of normal saline for 10 consecutive days followed by being exposed to a hypobaric hypoxic chamber simulating 5 000 m altitude for 48 h), DMF control (intraperitoneal injection of DMF for 10 consecutive days followed by being placed in a normobaric normoxic chamber), and DMF prophylaxis (DMF preconditioning for 10 d followed by hypobaric hypoxic modelling) groups. HE staining was used to observe the histopathological changes in lung tissues to pathologically score the lung injury. ELISA was employed to detect the inflammatory factors to assess inflammatory responses, immunofluorescence assay was utilized to measure the generation of reactive oxygen species (ROS), and spectrophotometry was performed to determine the glutathione (GSH) and malondialdehyde (MDA) levels, as well as superoxide dismutase (SOD) activity. Western blotting and real-time polymerase chain reaction (RT-qPCR) were applied to analyze ferroptosis-related pathway proteins at protein and mRNA levels. ② Hypoxic lung injury cellular model and normoxic control model were established by co-culturing human bronchial epithelial (BEAS-2B) cells and TNF-α-activated human peripheral blood monocyte (THP-1)-derived macrophages under hypoxic and normoxic conditions for 48 h. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to compare the protein profiles in BEAS-2B cells between normoxic control and hypoxic injury groups. Bioinformatics analysis was carried out to identify the key differentially expressed proteins (DEPs). A hypoxic injury prophylaxis group was established by BEAS-2B cells being pretreated with DMF for 24 h and then co-cultured with THP-1 cells. Then the ferroptosis markers, macrophage polarization phenotypes, and inflammatory cytokines were detected in BEAS-2B cells. ③ Additionally, solute carrier family 7 member 11 (SLC7A11) gene was knocked down under DMF intervention to assess ferroptosis markers and macrophage-related inflammatory responses. **Results** ① Compared to the hypoxic injury group, the DMF prophylaxis group exhibited significantly decreased pulmonary ROS level and MDA content (P<0.05), increased SOD activity and GSH level (P<0.05), elevated expression levels of SLC7A11 and glutathione peroxidase 4 (GPX4) (P<0.05), along with markedly reduced lung injury score and inflammatory cytokine levels (P<0.05). ② Proteomic analysis identified 5 377 proteins and 613 DEPs (302 up-regulated, 311 down-regulated) in BEAS-2B cells, and among 9 ferroptosis-related DEPs, SLC7A11 exhibited the most significant alteration. In the cellular hypoxic ALI model, compared to the hypoxic injury group, DMF prophylaxis up-regulated SLC7A11 and GPX4 expression (P<0.05), increased GSH level and

SOD activity, decreased the M1 macrophage ratio and inflammatory factor levels (P<0.05). ③ SLC7A11 knockdown reversed DMF's effects by increasing MDA level (P <0.05), decreasing SOD activity and GSH level (P<0.05), suppressing GPX4 expression (P<0.05), and elevating M1 macrophage proportion and enhancing inflammatory cytokine secretion (P<0.05). Conversely, overexpression of SLC7A11 enhanced the protective effects of DMF. Compared with the hypoxia-DMF group, the hypoxia-DMF-overexpression group showed significantly increased GSH level, SOD activity, and GPX4 expression (P<0.05), along with a marked decrease in MDA content (P<0.05) in BEAS-2B cells. Meanwhile, SLC7A11 overexpression further alleviated the inflammatory response, as indicated by significantly reduced expression of IL-1β, IL-6 and TNF-α (P<0.05). **Conclusion** DMF attenuates high-altitude hypoxic ALI by suppressing ferroptosis in pulmonary epithelial cells *via* up-regulating the NRF2/SLC7A11 axis, modulating macrophage polarization, and mitigating inflammatory response, and thus, exerts a protective effect against hypoxic pulmonary injury.

[**Key words**] high-altitude hypoxia; lung injury; ferroptosis; inflammatory response; macrophage; NRF2/SLC7A11

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高原指海拔2 500 m及以上的地区,其核心环境特征为缺氧。缺氧可诱发系统性生理功能紊乱[1],典型临床表现包括呼吸困难、胸闷、发绀及运动耐量下降,若症状未及时缓解,则会出现高原低氧导致的急性肺组织损伤与呼吸功能障碍[2]。高原低氧肺损伤以急性炎症反应为特征,引发肺泡上皮和肺血管内皮结构破坏[3],若不及时干预可进展为危害生命的高原肺水肿。当前临床干预主要依赖氧疗及梯度下撤等对症治疗措施[4],尚缺乏靶向核心病理机制的预防性药物。在低氧环境下,炎症细胞可进入肺泡间隙引发肺部炎症反应,现有研究表明高原低氧肺损伤的发病机制涉及炎症级联反应[5]、氧化应激失衡[6]及肺毛细血管高通透性[7]等分子病理过程。本课题组前期研究发现铁死亡在高原低氧肺损伤(acute lung injury,ALI)中具有重要意义,抑制铁死亡可减轻高原低氧ALI炎症反应[8],然而关于铁死亡在高原低氧ALI中的调控分子机制仍不清楚,有待进一步深入研究。

核因子E2相关因子2(nuclear factor erythroid 2-related factor 2,NRF2)作为关键性转录调节因子,其主要功能是在氧化应激状态下与靶基因启动子/增强子区域的抗氧化反应元件(antioxidant response element,ARE)结合,从而启动抗氧化防御机制,并在多种病理状态下参与铁死亡的调控[9]。研究证实,NRF2-ARE复合物的形成可显著增强包括溶质载体家族7成员11(solute carrier family 7 member 11,SLC7A11)和谷胱甘肽过氧化物酶4(glutathione peroxidase 4,GPX4)在内的重要抗氧化基因的表达,促进毒性脂质过氧化物向非活性脂质醇的转化,由此可重建细胞的氧化还原平衡[10-12]。有证据显示,缺氧条件能够通过降低NRF2的表达水平来促进铁死亡的发生;例如在心肌缺血缺氧过程中,心肌细胞内的钙超载引起线粒体功能紊乱,导致活性氧(reactive oxygen species,ROS)聚集,进而通过芬顿反应(Fenton reaction)抑制NRF2活性,最终引发铁死亡[13]。然而,在高原低氧环境诱导的肺损伤模型中,NRF2对铁死亡的潜在调控作用仍有待阐明。

SLC7A11作为系统Xc^-的关键功能亚基,其通过谷胱甘肽(glutathione,GSH)依赖的GPX4通路清除脂质过氧化物,防止铁依赖性脂质过氧化物的积累,由此抵御铁死亡的发生[14]。已有证据表明,缺氧条件下铁死亡的发生与SLC7A11存在紧密联系,低氧状态能够调节SLC7A11的转录水平进而调控铁死亡进程[15]。NRF2/SLC7A11信号轴参与了多种病理情况下的铁死亡调控,涵盖肺、脑、心脏及肝脏的缺血再灌注损伤,以及恶性肿瘤等[16-20]。具体而言,在心肌缺血再灌注损伤模型中,激活NRF2/SLC7A11/GPX4通路可减轻心肌细胞的铁死亡损伤,促进心脏功能恢复[14];而在脂多糖(lipopolysaccharide, LPS)或肠道缺血-再灌注诱导的急性肺损伤中,NRF2的活化可上调SLC7A11表达,有效抑制肺泡上皮细胞的铁死亡[21]。然而,鉴于铁死亡在炎症调节中的潜在作用,针对低氧环境下NRF2是否通过调节SLC7A11影响人支气管上皮(human bronchial epithelial,BEAS-2B)细胞铁死亡,进而调控巨噬细胞极化状态及其介导的炎症级联反应,相关机制研究仍较匮乏。

富马酸二甲酯(dimethyl fumarate,DMF)是目前最成功的NRF2激动剂[22]。研究证实,DMF在动物模型和人体实验中均可激活NRF2通路,药物应用可促进NRF2蛋白稳定化,激活NRF2依赖性转录活性,从而

上调NRF2表达水平[22]。有研究发现慢性阻塞性肺疾病(chronic obstructive pulmonary disease,COPD)患者肺组织NRF2抗氧化防御通路显著衰退,肺组织抵御COPD氧化应激损伤的能力下降[23]。激活NRF2通路能上调抗氧化防御机制、缓解肺部炎症并减少肺泡细胞凋亡[24]。此外,NRF2激动剂(CDDO-Me、CDDO-Im)通过提升NRF2活性对小鼠急性肺损伤模型产生强效保护作用,白藜芦醇亦通过激活Nrf2保护人肺上皮细胞免受氧化应激损伤[23]。目前,DMF仅作为FDA批准作为治疗复发型多发性硬化症的一线口服药物[25],其是否能减轻高原低氧肺损伤炎症反应还有待研究。

本研究基于实验室前期已经成功构建的高原低氧ALI动物模型和细胞共培养模型[8],系统性验证NRF2/SLC7A11轴在高原低氧肺损伤铁死亡中的作用,并进一步阐明NRF2激动剂DMF对高原低氧ALI的保护作用,为高原低氧ALI患者提供新的潜在预防方案。

1 材料与方法

1.1 实验动物与细胞

20只SPF级雄性Wistar大鼠(体质量210~230 g,5~6周龄)由解放军总医院动物实验中心提供[许可证号:SCXK-(JUN)2012-0004]。所有动物于温度(24±1)℃、相对湿度(50±5)%、12 h/12 h明暗循环条件下适应性饲养3~5 d。本实验方案经解放军总医院医学伦理委员会批准(S2021-623-01)。人肺上皮细胞BEAS-2B系及人类外周血单核细胞(tumor human peripheral blood monocytes-1,THP-1)系购自武汉普诺赛生命科技有限公司,均通过短串联重复序列(short tandem repeats,STR)鉴定。

1.2 实验方法

1.2.1 动物处理及分组 将20只大鼠按随机数字表法分为4组(n=5):常氧对照组、低氧肺损伤组、DMF对照组和DMF预防组。所有组别大鼠连续10 d每日1次腹腔注射相应药物:常氧对照组和低氧肺损伤组注射生理盐水;DMF对照组和DMF预防组注射DMF(上海MCE公司,4 mg/kg)。末次注射24 h后,将常氧对照组和DMF对照组大鼠置于常氧条件下正常饲养48 h;将低氧肺损伤组和DMF预防组大鼠置于模拟海拔5 000 m的低压低氧舱中暴露48 h。氧舱以恒定速度0.5 m/s升降,模拟海拔变化。舱内氧浓度稳定于10%~13%,符合高原缺氧特征。实验全程保证大鼠自由饮水和进食,舱内及实验室环境温度维持在(20±2)℃,湿度保持在50%~60%。低氧暴露48 h后,将氧舱逐步恢复至常压常氧条件。本研究设定明确的人道终点(如体质量下降超过20%或出现不可逆呼吸困难)。实验动物的健康状况由专业人员每日早、晚各监测一次,如大鼠达到人道终点,将立即腹腔注射戊巴比妥钠(150 mg/kg)实施安乐死以减轻其痛苦。

1.2.2 细胞处理及分组 将BEAS-2B细胞和巨噬细胞(由THP-1诱导而来)分为7组:常氧对照组(两种细胞共培养后置于常氧环境下共培养48 h)、DMF对照组(DMF+常氧共培养48 h)、低氧肺损伤组(两种细胞共培养后置于低氧培养箱中培养48 h)、DMF预防组(DMF预处理BEAS-2B细胞24 h后与巨噬细胞低氧共培养48 h)、DMF预防敲低组1(oligo1敲低BEAS-2B细胞SLC7A11后,给予DMF预处理24 h,随后与巨噬细胞低氧共培养48 h)、DMF预防敲低组2(oligo2敲低BEAS-2B细胞SLC7A11后,给予DMF预处理24 h,随后与巨噬细胞低氧共培养48 h)和DMF预防过表达组(过表达BEAS-2B细胞SLC7A11后,给予DMF预处理24 h,随后与巨噬细胞低氧共培养48 h)。SLC7A11 oligo1/oligo2、SLC7A11过表达质粒均购自吉玛基因公司。

采用100 μL PMA(上海MCE公司,10 μg/mL)处理THP-1细胞24 h,使其极化为贴壁状态的M0型巨噬细胞。为建立体外低氧肺损伤模型,利用氮气置换氧气将培养箱内O_2浓度降至1%(CO_2保持5%)。随后,采用Transwell共培养系统进行低氧共培养:将BEAS-2B细胞接种于六孔板底室,将PMA诱导分化的M0型巨噬细胞置于Transwell上室,在1%O_2、5%CO_2条件下共培养48 h,此组即为低氧肺损伤组。以20 μmDMF预处理BEAS-2B细胞24 h,更换新鲜培养基后再将两种细胞在常氧和低氧条件下分别共培养48 h作为DMF对照组和DMF预防组。

采用siRNA敲减BEAS-2B细胞中SLC7A11表达,细胞铺板贴壁24 h后,使用GP-Transfect-Mate转染试剂(吉玛基因公司)及特异性siRNA-SLC7A11进行转染,转染6 h后更换完全培养基,随后加入20 μmol/L DMF处理24 h。再与M0型巨噬细胞在1%O_2、5%CO_2条件下共培养48 h,作为DMF预防敲减组进行后续实验。

采用过表达质粒过表达BEAS-2B细胞中SLC7A11表达,细胞铺板贴壁24 h后,使用lipomaster 3000 Transfection Reagent(诺唯赞公司)进行转染,转染6 h后更换完全培养基,随后加入20 μmol/L DMF处理24 h。再与M0型巨噬细胞在1%O_2、5%CO_2条件下共培养48 h,作为DMF预防过表达组进行后续实验。

1.2.3 肺组织病理学观察 造模完成后,将各组大鼠腹腔注射巴比妥钠(150 mg/kg)处死,取肺组织,将大鼠肺组织于4%多聚甲醛中固定,经梯度浓度乙

醇脱水、二甲苯透明化处理及石蜡包埋后切片,行HE染色观察各组肺组织病理学变化并进行损伤评分。依据HE染色结果,每组选取5~8个视野进行肺损伤Smith评分:针对肺水肿、肺泡间质炎症、肺不张与透明膜形成4项指标,按病变累及范围分级计分(病变范围<25%计1分,25%~<50%计2分,50%~<75%计3分,≥75%计4分),最终计算同组视野评分的均值作为该组肺损伤程度量化指标。

1.2.4 免疫荧光检测 大鼠肺组织样本经冰冻切片制备后,室温干燥,滴加ROS特异性荧光探针(日本Sigma公司),37 ℃避光孵育30 min。随后经PBS漂洗3次(每次5 min),烘干后滴加DAPI核染液复染,室温避光处理10 min。同法PBS漂洗3次后晾干切片,采用含抗淬灭剂的封片介质封固,于荧光显微镜下观察并采集图像。

1.2.5 GSH含量测定 按碧云天GSH含量检测试剂说明书进行操作。大鼠肺组织样本经液氮研磨后,4 ℃静置10 min,于4 ℃以14 000×*g*离心分离10 min,收集上清液用于总GSH检测。对于BEAS-2B细胞沉淀,加入蛋白脱除溶液重悬混匀,依次经液氮速冻与37 ℃水浴解冻循环处理2次以实现充分裂解,在4 ℃下以14 000×*g*离心10 min后取上清作为GSH检测样本。将GSH母液用蛋白脱除溶液梯度稀释(浓度梯度:10.0、5.0、2.0、1.0、0.5 μmol/L)制备标准曲线溶液。检测体系设置如下:每孔加入10 μL样本或标准品,添加150 μL反应工作液,室温孵育5 min后注入50 μL 0.5 mg/mL NADPH溶液。混合反应25 min后,采用酶标仪于412 nm波长测定吸光度[*D*(412)]值。依据标准品梯度浓度与吸光度的对应关系建立回归方程,据此计算样本总GSH含量。

1.2.6 丙二醛(malondialdehyde,MDA)含量测定

按碧云天MDA含量检测试剂说明书进行操作。精确称取20 mg大鼠右肺组织样本,经液氮冷冻粉碎后,加入500 μL预稀释的抗氧化剂(Antioxidant)溶液继续匀浆处理。所得匀浆混合液在4 ℃条件下以10 000×*g*离心5 min,收集200 μL上清作为待测样本。随后利用上海东仁化学科技有限公司生产的丙二醛(MDA)检测试剂盒,严格遵循制造商操作指南构建反应体系并进行比色法测定。

1.2.7 超氧化物歧化酶(super oxide dismutase,SOD)活性测定 按碧云天SOD活性检测试剂说明书进行操作。取大鼠肺组织样本经液氮冷冻粉碎后制备组织匀浆,通过超声细胞破碎仪(功率60 W,脉冲间隔0.5 s)裂解15 min,随后10 000×*g*、4 ℃离心10 min,获取上清液作为检测样本。对于细胞样本,收集细胞沉淀后经2 000×*g*、4 ℃低温离心10 min,去除上清,PBS清洗2次。细胞沉淀经超声破碎处理(参数:功率100 W,脉冲间隔5 s,持续10 min),10 000×*g*、4 ℃离心15 min,收集上清液待测。所有样本的SOD活性测定采用上海东仁化学科技有限公司生产的SOD检测试剂盒,严格遵循制造商技术规范进行操作。

1.2.8 流式细胞术检测巨噬细胞CD86和CD206表达情况 通过气管切口插入钝头套管,分次缓慢灌注并回收预冷无菌PBS(1 mL/100 g体质量,含EDTA/BSA),重复2~3次,合并回收液以获取大鼠支气管肺泡灌洗液(bronchoalveolar lavage fluid, BALF),立即离心分离获取细胞沉淀,用PBS制备单细胞悬液,加入5 μL CD86与CD206荧光抗体避光孵育15 min,随后1 500 r/min常温离心5 min。弃上清,再用1 mL PBS洗1遍,重复3次,采用美国BD FACS Aria Ⅱ型流式细胞仪进行荧光分析。

1.2.9 CCK8检测药物对细胞活力的影响 将BEAS-2B细胞稀释至80个/μL,以100 μL/孔种至96孔板中,100 μL/孔培养基作为空白孔。BEAS-2B细胞贴壁24 h后吸出培养基,加入用完全培养基稀释的DMF,浓度分别为0、2.5、5.0、10.0、20.0、40.0、80.0 μmol/L,每个浓度设置5个副孔。将96孔板放回培养箱中继续培养48 h后将CCK8溶液以10 μL/孔加入96孔板,再放回培养箱中继续培养2 h,用酶标仪检测450 nm的吸光度[*D*(450)]值。细胞活力=($D(450)_{加药组}-D(450)_{空白组}$)×100/($D(450)_{未加药组}-D(450)_{空白组}$)。

1.2.10 液相色谱-串联质谱法(liquid chromatography-tandem mass spectrometry,LC-MS/MS)分析 BEAS-2B细胞蛋白质组学分析采用EASY-nLC1200纳米系统与Orbitrap结合使用的Exploris480质谱仪(ThermoScientific,SanJose,CA)。数据依赖采集(data-dependent acquisition,DDA)采用75 min梯度洗脱,参数设置如下:电喷雾电压2.2 kV,扫描范围350~1 500 m/z;补偿电压-45 V或-65 V以确保质量精度。MS1与MS2分辨率分别设为60 000和15 000,归一化自动增益控制(automatic gain control, AGC)目标值分别为300%和75%。MS1最大注入时间50 ms,MS2最大注入时间22 ms。隔离窗口宽度1.6 m/z,归一化碰撞能量27%。

1.2.11 生物信息学分析 蛋白质组学数据分析采用Proteome Discoverer软件(Thermo Fisher,v2.2)的Sequest HT算法解析原始数据,并通过UniProt人类数据库进行肽段鉴定。原始蛋白表达数据经Perseus软件实施归一化、对数转换及正态分布检验等预处理。

设定错误发现率(false discovery rate, FDR)<1%且肽段可信度评分>40作为筛选标准,同时排除在任一实验组中缺失率>80%的蛋白质。差异表达蛋白的筛选依据校正后*P*值(q)<0.05及差异倍数(fold change, FC)>1.5或<0.67的标准进行双侧*t*检验。针对组间筛选的差异表达蛋白(differentially expressed proteins, DEPs),利用Oebiotech云平台(https://cloud.oebiotech.cn)绘制热图、火山图与偏最小二乘判别分析(partial least squares discriminant analysis, PLS-DA)图,并实施基于基因本体论(gene ontology, GO)的生物过程富集分析和基于京都基因与基因组百科全书(Kyoto encyclopedia of genes and genomes, KEGG)的通路富集分析。

1.2.12 总RNA提取和RT-qPCR 大鼠肺组织样本经液氮研磨后,与RL裂解缓冲液混合涡旋匀浆,细胞样本经离心收集沉淀后同法裂解。采用RNA提取试剂盒提取总RNA,使用Nanodrop分光光度计测定RNA浓度。随后依据反转录试剂盒说明书合成cDNA,并将稀释后的cDNA产物用于*Taq* Pro Universal SYBR qPCR Master Mix反应体系的构建。引物设计参考NCBI数据库(https://www.ncbi.nlm.nih.gov/)收录的大鼠基因序列,并由生工生物工程(上海)股份有限公司合成,扩增反应在实时定量PCR系统上完成。

1.2.13 Western blot检测蛋白表达水平 大鼠肺组织称量后与细胞样本分别经裂解液处理,4 ℃条件下12 000×*g*离心20 min获取上清。采用BCA法进行蛋白浓度定量并统一上样量,经10%或12%SDS-PAGE电泳分离后转至PVDF膜。5%脱脂奶粉封闭1 h,先后加入NRF2、SLC7A11、GPX4、FTH1、IL-1、IL-6、TNF-α及β-actin一抗(4 ℃孵育过夜)和HRP标记二抗(室温避光孵育1 h)。ECL化学发光显影后通过凝胶成像系统采集图像,使用Image J软件分析目标蛋白与β-actin内参的条带灰度值,计算相对表达量。

1.2.14 ELISA检测巨噬细胞分泌的炎症因子 收集巨噬细胞培养上清液,4 ℃条件下12 000×*g*离心15 min去除细胞碎片。使用商品化ELISA试剂盒,将标准品和样本分别加入预包被抗体的96孔板,37 ℃孵育90 min。PBS洗涤后加入生物素标记检测抗体,37 ℃孵育60 min,再次洗涤并加入链霉亲和素-HRP复合物,37 ℃避光反应30 min。洗涤后加入TMB底物溶液进行显色反应,室温避光孵育15 min,最后加入终止液终止反应。即刻于450 nm波长测定吸光度[*D*(450)]值,根据标准曲线计算IL-1β、IL-6、TNF-α等炎症因子的相对浓度,所有样本设复孔检测。

1.3 统计学分析

所有统计数据通过GraphPad Prism 9.0.0软件进行处理与分析,结果以可视化图表形式呈现。符合正态分布的计量数据以$\bar{x}\pm s$表示,多组间连续变量比较采用单因素方差分析(One-way ANOVA),组间两两比较辅以Tukey事后检验;两组间差异评估采用双尾Student's *t*检验。以*P*<0.05为差异有统计学意义。

2 结果

2.1 DMF上调NRF2抑制高原低氧肺损伤大鼠肺组织铁死亡的发生

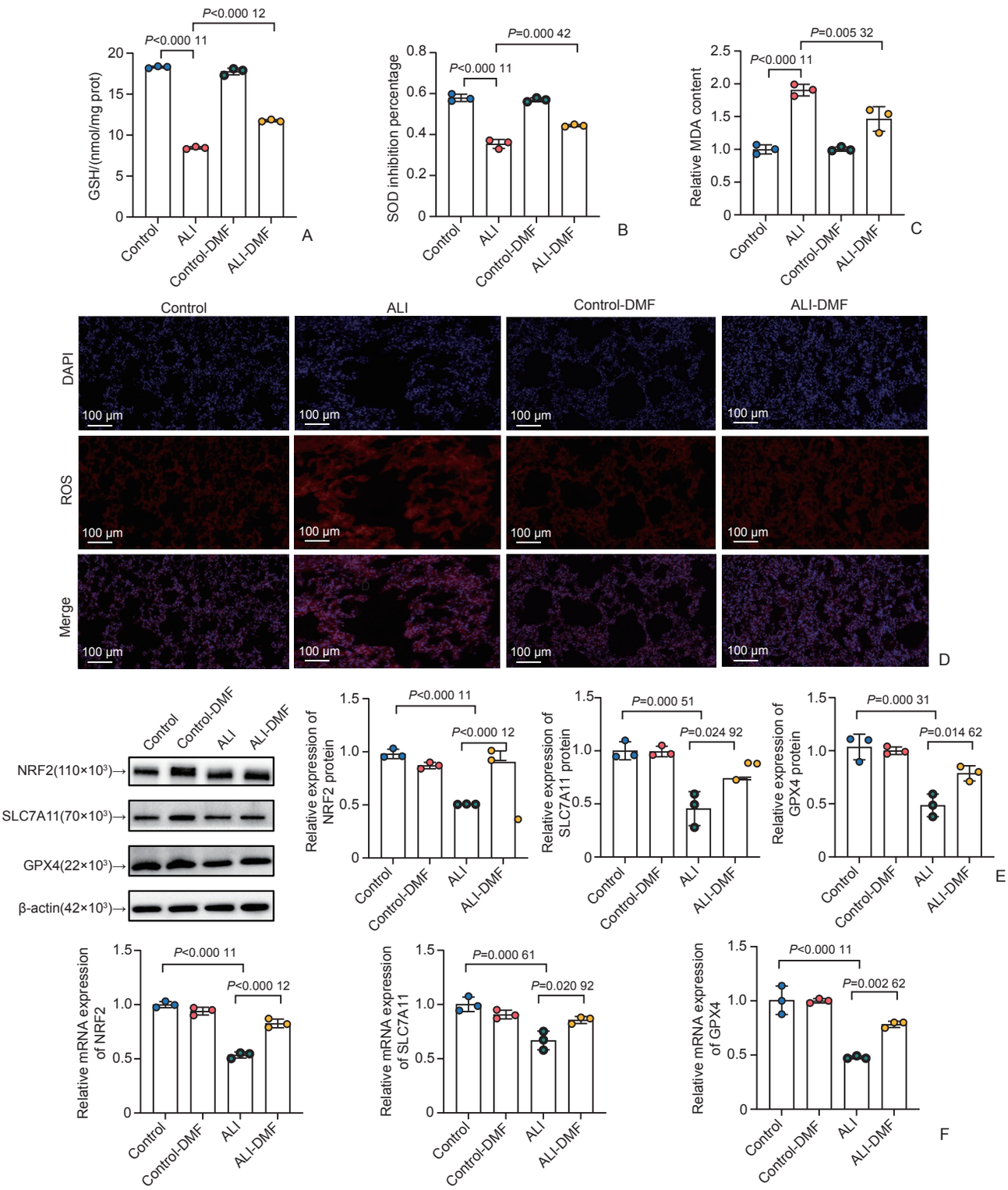
给予NRF2激动剂DMF观察是否能抑制高原低氧肺损伤中铁死亡的发生,以验证NRF2对铁死亡的调控作用。在给予DMF后,DMF预防组大鼠肺组织GSH水平、SOD活性相较于低氧肺损伤组均显著上升(*P*<0.05,图1A、B),而MDA含量和ROS水平相较于低氧肺损伤组降低(*P*<0.05,图1C、D),提示DMF通过上调NRF2的表达减轻低氧肺损伤组大鼠肺组织的脂质过氧化水平。除此之外,给予DMF后,与低氧肺损伤组相比,DMF预防组NRF2升高,SLC7A11、GPX4表达也随之升高(*P*<0.05,图1E),mRNA水平与蛋白水平变化一致(*P*<0.05,图1E)。这提示DMF通过上调NRF2的表达,改善低氧肺损伤大鼠肺组织脂质过氧化失衡,从而调控高原低氧肺损伤大鼠肺组织铁死亡。

2.2 DMF上调NRF2改善低氧诱导的急性肺损伤大鼠肺组织炎症反应

大鼠肺组织HE染色结果提示给予DMF后,DMF预防组相较于高原低氧肺损伤组肺损伤症状有所缓解,肺损伤评分显著降低(*P*<0.05,图2A)。此外,DMF处理后,DMF预防组相较于高原低氧肺损伤组大鼠肺组织中促炎因子IL-6、TNF-α表达显著降低(*P*<0.05,图2B),支气管灌洗液中$CD86^+$M1型巨噬细胞比例显著降低(*P*<0.05,图2C),而$CD206^+$M2型巨噬细胞比例显著升高(*P*<0.05,图2C)。除此之外,DMF给药后,部分逆转了M1型巨噬细胞iNOS表达的升高和M2型巨噬细胞Arg-1表达的降低(*P*<0.05,图2D)。在课题组前期工作中,我们发现低氧肺损伤中铁死亡的发生,给予铁死亡抑制剂后,铁死亡显著改善的同时,低氧肺损伤炎症反应也显著减轻,这提示抑制铁死亡可缓解低氧肺损伤炎症反应[8]。综上提示DMF通过上调NRF2改善大鼠肺组织铁死亡,从而抑制高原低氧肺损伤炎症反应。

2.3 DMF上调NRF2减轻BEAS-2B细胞铁死亡

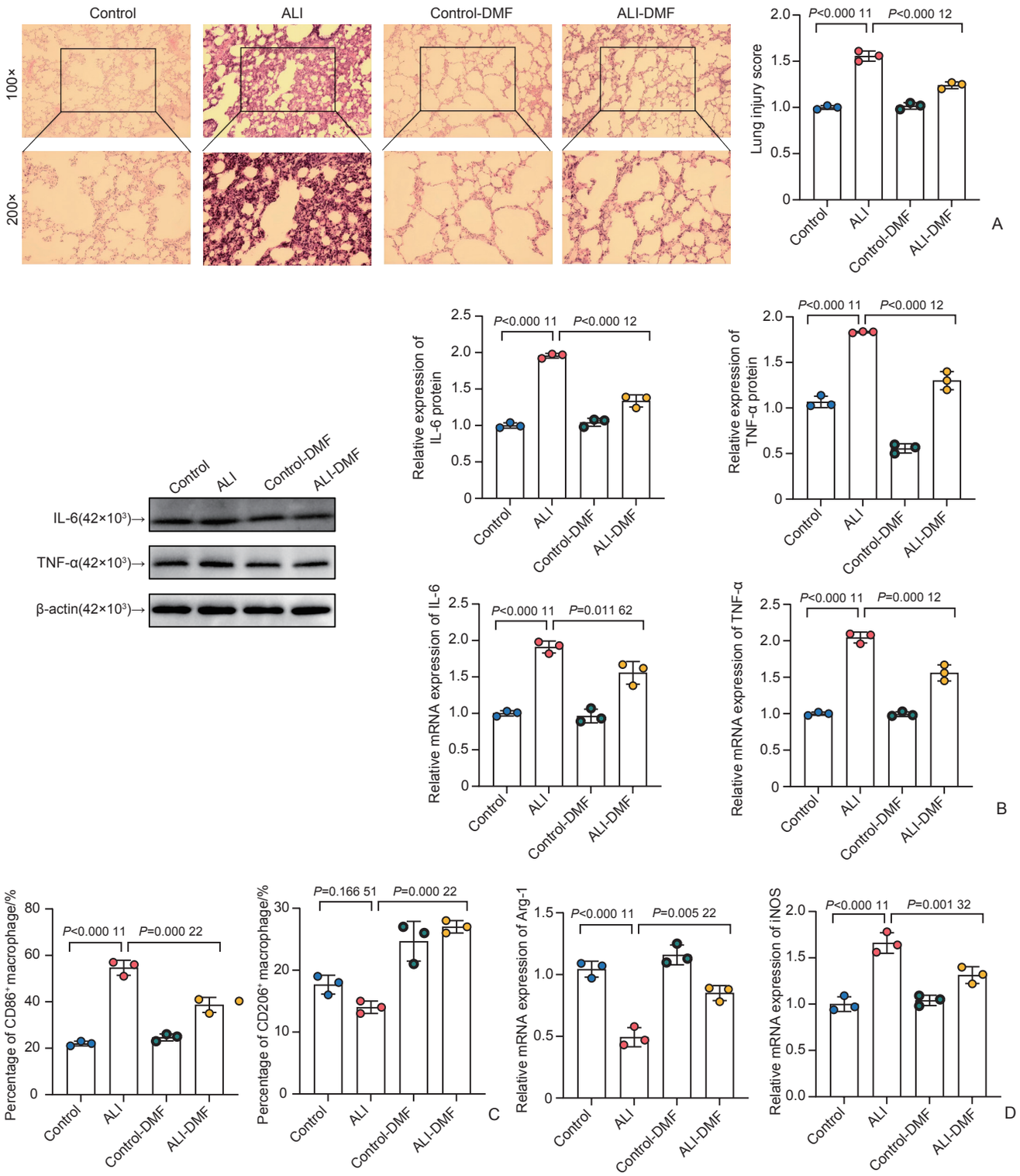
低氧暴露后,BEAS-2B细胞与巨噬细胞的活力均随时间延长呈依赖性下降(图3A);通过CCK8实验确定DMF的最适给药浓度为20 μmol/L(图3B)。免疫荧



A~C: Histogram of GSH level, SOD activity and MDA content in lung tissues from the Control, Control-DMF, ALI and ALI-DMF groups; D: Representative fluorescence images showing ROS expression in lung tissues from each group. E, F: Protein and mRNA expression levels of NRF2, SLC7A11 and GPX4 in lung tissues from each group. DMF: Dimethyl fumarate; ALI: Acute lung injury; GSH: Glutathione; MDA: Malondialdehyde; SOD: Superoxide dismutase; ROS: Reactive oxygen species; NRF2: Nuclear factor erythroid 2-related factor 2; SLC7A11: Solute carrier family 7 member 11; GPX4: Glutathione peroxidase 4

图1 DMF上调NRF2对高原低氧肺损伤大鼠肺组织铁死亡的抑制作用(n=5,$\bar{x}\pm s$)

Figure 1 DMF attenuates ferroptosis in lung tissue of rats with high-altitude hypoxia-induced lung injury *via* up-regulation of NRF2 (n=5,$\bar{x}\pm s$)

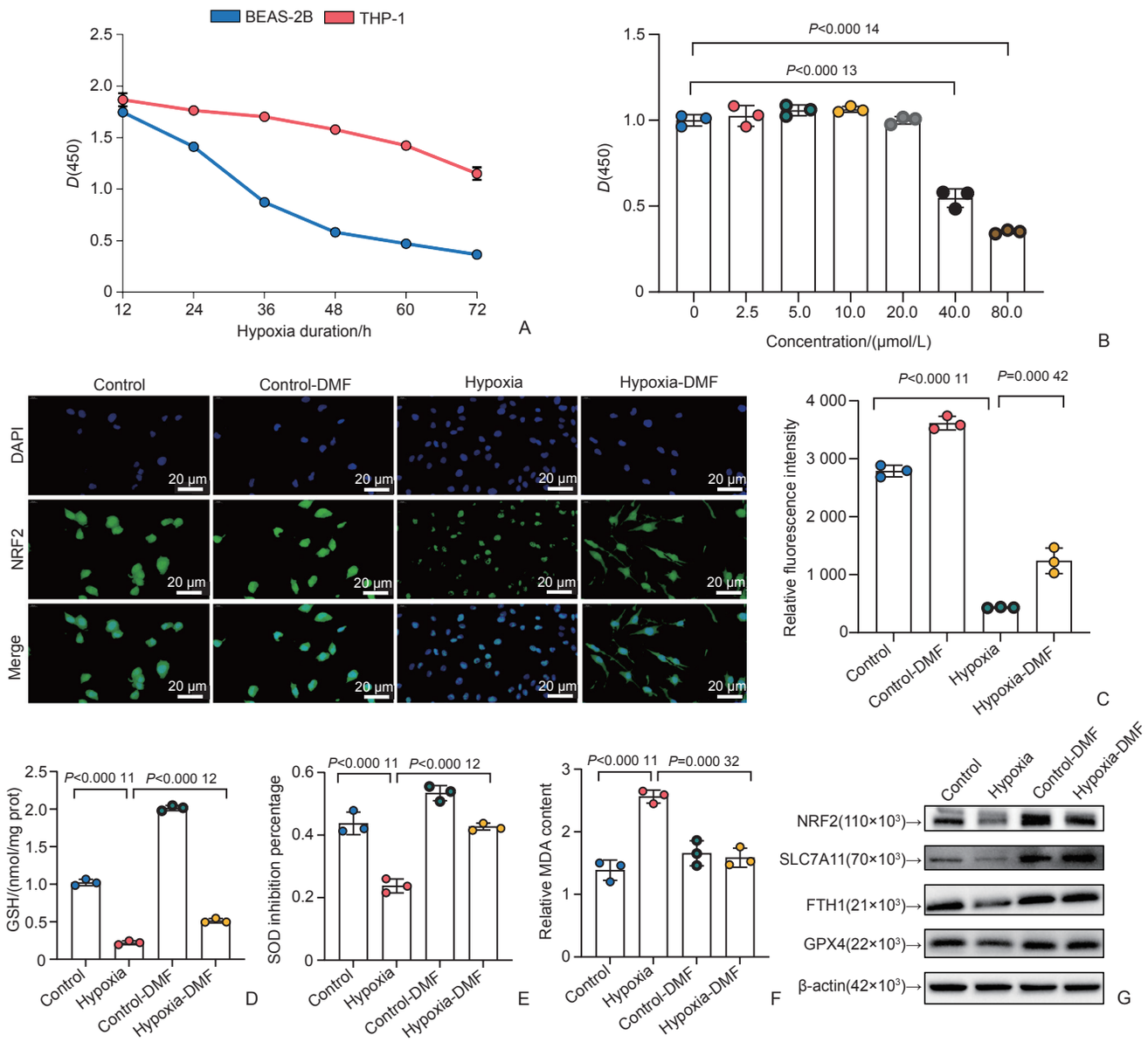


A: Histopathological changes (HE staining) and lung injury scores in lung tissues from the Control, Control-DMF, ALI and ALI-DMF groups; B: Protein and mRNA expression levels of inflammatory cytokines IL-6 and TNF-α in lung tissues from each group; C: Macrophage polarization in bronchoalveolar lavage fluid from the normoxic control, hypoxia-induced lung injury, and DMF prophylaxis groups; D: mRNA expression of macrophage polarization markers Arg-1 and iNOS in lung tissues from each group

图2 DMF上调NRF2缓解高原低氧诱导的急性肺损伤大鼠肺组织炎症反应(n=5,$\bar{x}\pm s$)

Figure 2 DMF alleviates inflammatory response in lung tissue of rats with high-altitude hypoxia-induced acute lung injury *via* upregulation of NRF2 (n=5,$\bar{x}\pm s$)

光检测显示，与常氧对照组相比，低氧损伤组BEAS-2B细胞中NRF2的表达显著降低，而DMF干预后其表达明显回升（$P<0.05$，图3C），Western blot实验结果进一步验证了这一趋势（$P<0.05$，图3H）。此外，较于常氧对照组，低氧肺损伤组BEAS-2B细胞内GSH水平和SOD活性降低（$P<0.05$，图3D、E），MDA含量显著升高（$P<0.05$，图3F）；在给予DMF后，DMF预防组GSH水平、SOD活性相较于低氧肺损伤组显著回升（$P<0.05$，图3D、E），而MDA含量显著降低（$P<0.05$，图3F）。与此同时，低氧肺损伤组相较于常氧对照组铁死亡负调控因子SLC7A11、GPX4和FTH1表达显著下降（$P<0.05$，图3G、H），经DMF预防性干预后，SLC7A11、GPX4和FTH1表达则明显恢复（$P<0.05$，图3G、H）。这些结果表明DMF通过上调NRF2表达缓解BEAS-2B细胞铁死亡。

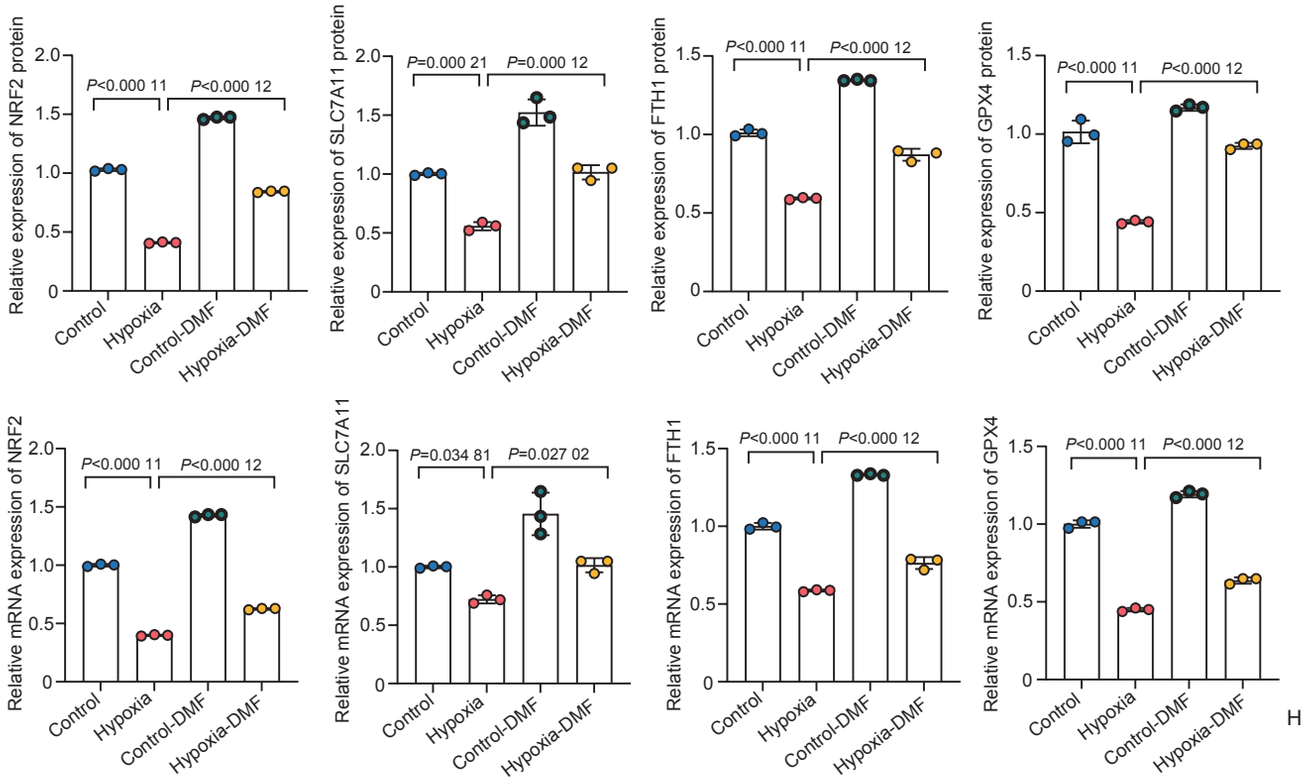


A: Viability of BEAS-2B and THP-1 cells after hypoxic exposure; B: Effects of different concentrations of DMF on BEAS-2B cell viability; C: Expression of NRF2 in BEAS-2B cells from the Control, Control-DMF, Hypoxia and Hypoxia-DMF groups; D~F: Histograms of GSH level (D), SOD activity (E), and MDA content (F) in BEAS-2B cells from each group; G: Western blotting results of NRF2, SLC7A11, FTH1, and GPX4 in BEAS-2B cells from each group

图3　DMF通过上调NRF2减轻BEAS-2B细胞铁死亡（$n=5$，$\bar{x}\pm s$）

Figure 3　DMF alleviates ferroptosis in BEAS-2B cells *via* upregulation of NRF2（$n=5$，$\bar{x}\pm s$）

（图3H续下页）



H: Protein and mRNA expression levels of NRF2, SLC7A11, FTH1, and GPX4 in BEAS-2B cells from each group

续图3 DMF通过上调NRF2减轻BEAS-2B细胞铁死亡(n=5,$\bar{x}\pm s$)

Figure 3(continued) DMF alleviates ferroptosis in BEAS-2B cells *via* upregulation of NRF2 (n=5,$\bar{x}\pm s$)

2.4 DMF上调BEAS-2B细胞NRF2改善低氧肺损伤中与之共培养的巨噬细胞炎症反应

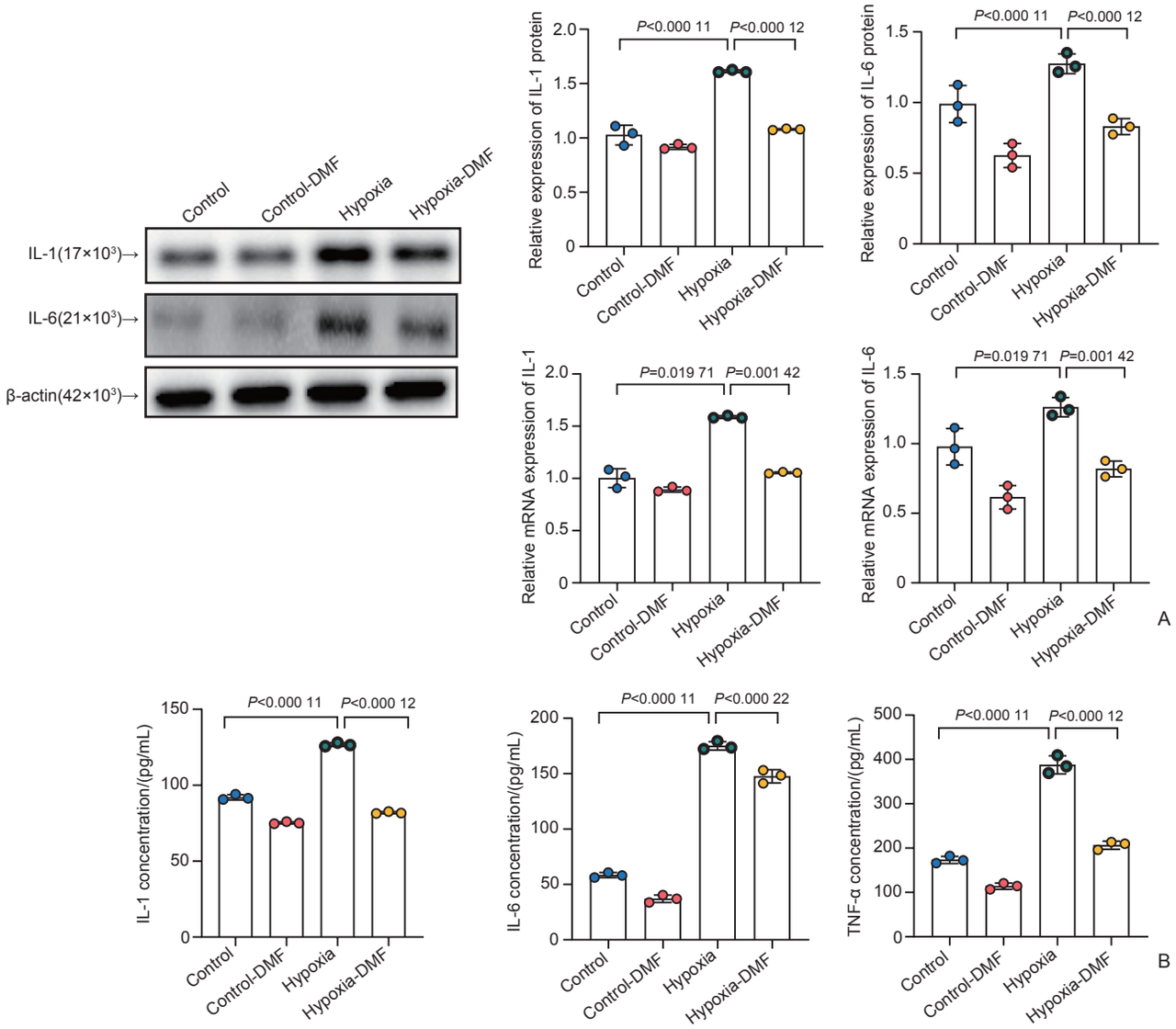
深入探究低氧肺损伤中NRF2调控铁死亡的具体机制,本研究采用BEAS-2B细胞与THP-1来源的巨噬细胞在低氧条件下共培养,以模拟体内肺损伤过程中巨噬细胞募集至损伤部位的微环境。结果显示,与常氧对照组相比,低氧肺损伤组巨噬细胞上清液中IL-1、IL-6和TNF-α的分泌水平均显著升高(P<0.05,图4B);而在对BEAS-2B细胞给予NRF2激动剂DMF干预后,DMF预防组中上述炎症因子的分泌水平均出现明显下降(P<0.05,图4B)。进一步地,在转录水平与蛋白水平上,低氧肺损伤组巨噬细胞中IL-1和IL-6的表达也较常氧对照组显著上调,而DMF处理则能有效抑制其表达(P<0.05,图4A)。以上结果表明,通过DMF上调BEAS-2B细胞的NRF2表达,改善其铁死亡,从而减轻由巨噬细胞介导的炎症反应。

2.5 BEAS-2B细胞蛋白组学提示SLC7A11为高原低氧肺损伤中的关键调控分子

在建立高原低氧肺损伤细胞共培养模型后,通过蛋白组学分析低氧肺损伤组和常氧对照组BEAS-2B细胞中的关键通路,BEAS-2B细胞蛋白组学共检测出2组5 377个蛋白,聚类分析显示差异蛋白可将常氧对照组和低氧肺损伤组进行明显区分(图5A),共筛选到613个差异表达蛋白,包括302个上调蛋白和311个下调蛋白(图5B)。KEGG分析富集到的通路包括胆固醇代谢、铁死亡、P53信号通路和TNF信号通路(图5C),这与本课题组前期研究在动物水平通过蛋白质组学分析富集到铁死亡通路的结果相一致(图5C)。为了进一步探索高原低氧肺损伤BEAS-2B细胞中铁死亡的关键调控分子,我们将BEAS-2B细胞蛋白组学中的613个差异表达蛋白与FerrDb V2数据库(https://ferrdb.org)中101个铁死亡相关蛋白取交集,得到9个重叠蛋白,分别是ALB、SLC3A2、HBA1、VLDLR、BNIP3、SRXN1、FTH1、SLC7A11和TFRC(图5D),其中SLC3A2和SLC7A11表达在常氧对照组与低氧肺损伤组中变化相对明显(图5E),提示SLC7A11可能在高原低氧肺损伤中的关键调控分子。

2.6 DMF通过NRF2/SLC7A11轴抑制高原低氧肺损伤BEAS-2B细胞铁死亡的发生

在给予细胞DMF后,DMF预防组BEAS-2B细胞MDA水平显著降低;然而siRNA敲减SLC7A11后部分逆转了DMF的作用,DMF预防敲低组相较于DMF预



A: Protein and mRNA expression levels of IL-1 and IL-6 in macrophages under hypoxic lung injury conditions after NRF2 upregulation; B: Secretions of IL-1, IL-6 and TNF-α in macrophage culture supernatant under hypoxic lung injury conditions after NRF2 upregulation

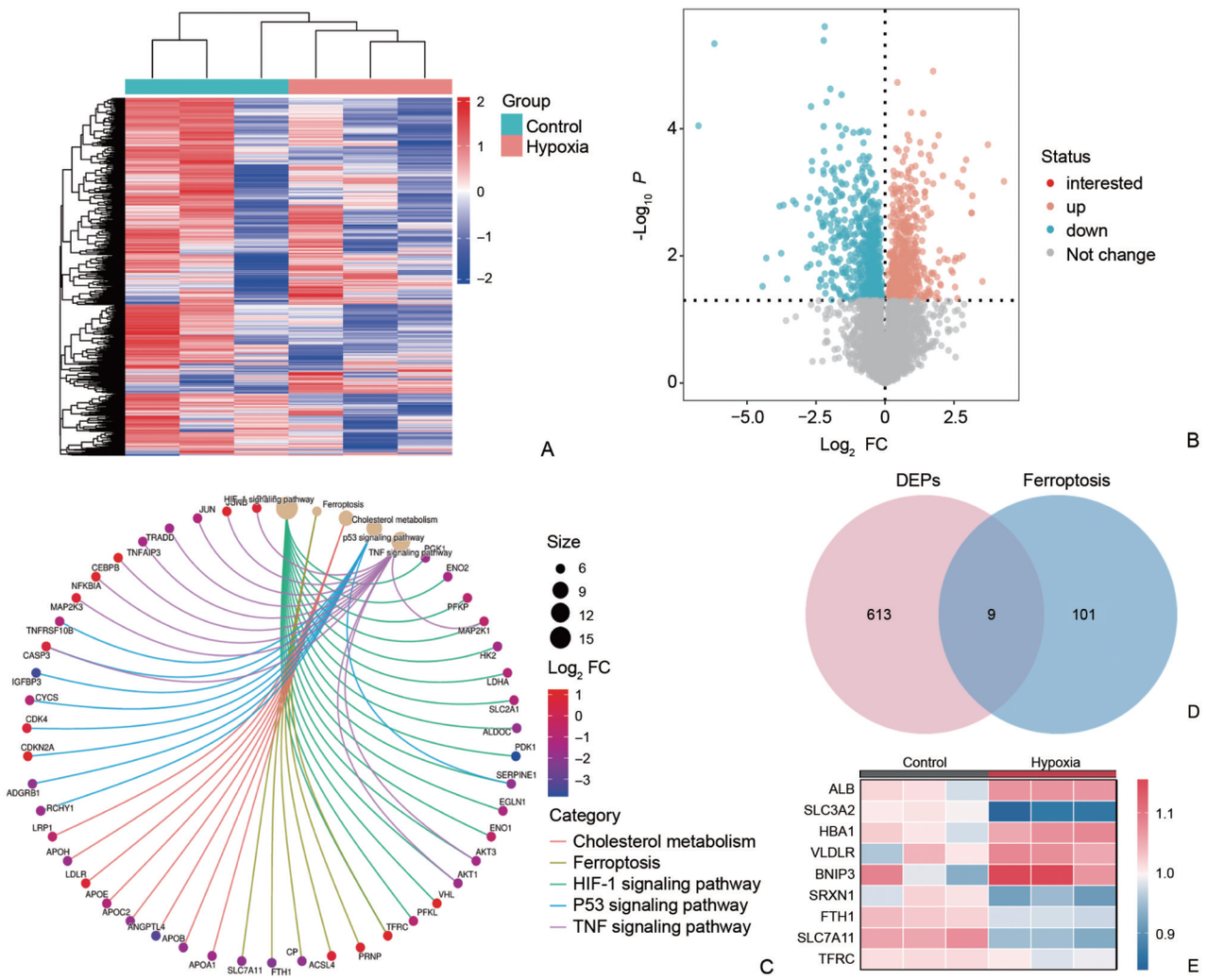
图4 DMF通过上调NRF2减轻低氧肺损伤巨噬细胞介导的炎症反应(n=5,$\bar{x}\pm s$)

Figure 4 DMF alleviates macrophage-mediated inflammatory response in hypoxic lung injury *via* upregulation of NRF2(n=5,$\bar{x}\pm s$)

防组BEAS-2B细胞GSH水平和SOD活性显著降低,而MDA含量显著升高(P<0.05,图6A)。相反,过表达的SLC7A11进一步增强了DMF的作用,DMF预防过表达组相较于DMF预防组BEAS-2B细胞的GSH水平和SOD活性显著升高,而MDA含量显著降低(P<0.05,图6B)。这些结果表明,过表达SLC7A11可进一步增强DMF的保护作用,而敲低SLC7A11则部分逆转了NRF2上调对脂质过氧化的缓解效应。此外,在DMF处理基础上敲低SLC7A11部分削弱了DMF的保护作用,DMF预防敲低组相较于DMF预防组SLC7A11和GPX4表达显著下降(P<0.05,图6C),而过表达SLC7A11后,DMF预防过表达组中SLC7A11与GPX4表达进一步升高(P<0.05,图6D)。综上结果提示,在低氧肺损伤细胞模型中,DMF通过NRF2/SLC7A11轴缓解BEAS-2B细胞脂质过氧化,提高细胞铁死亡负向调控蛋白的表达来抑制BEAS-2B细胞铁死亡。

2.7 DMF通过激活NRF2/SLC7A11轴来抑制高原低氧肺损伤巨噬细胞的炎症反应

NRF2是SLC7A11的典型上游调控分子[26],而SLC3A2是SLC7A11的伴侣蛋白[27],主要功能是维持

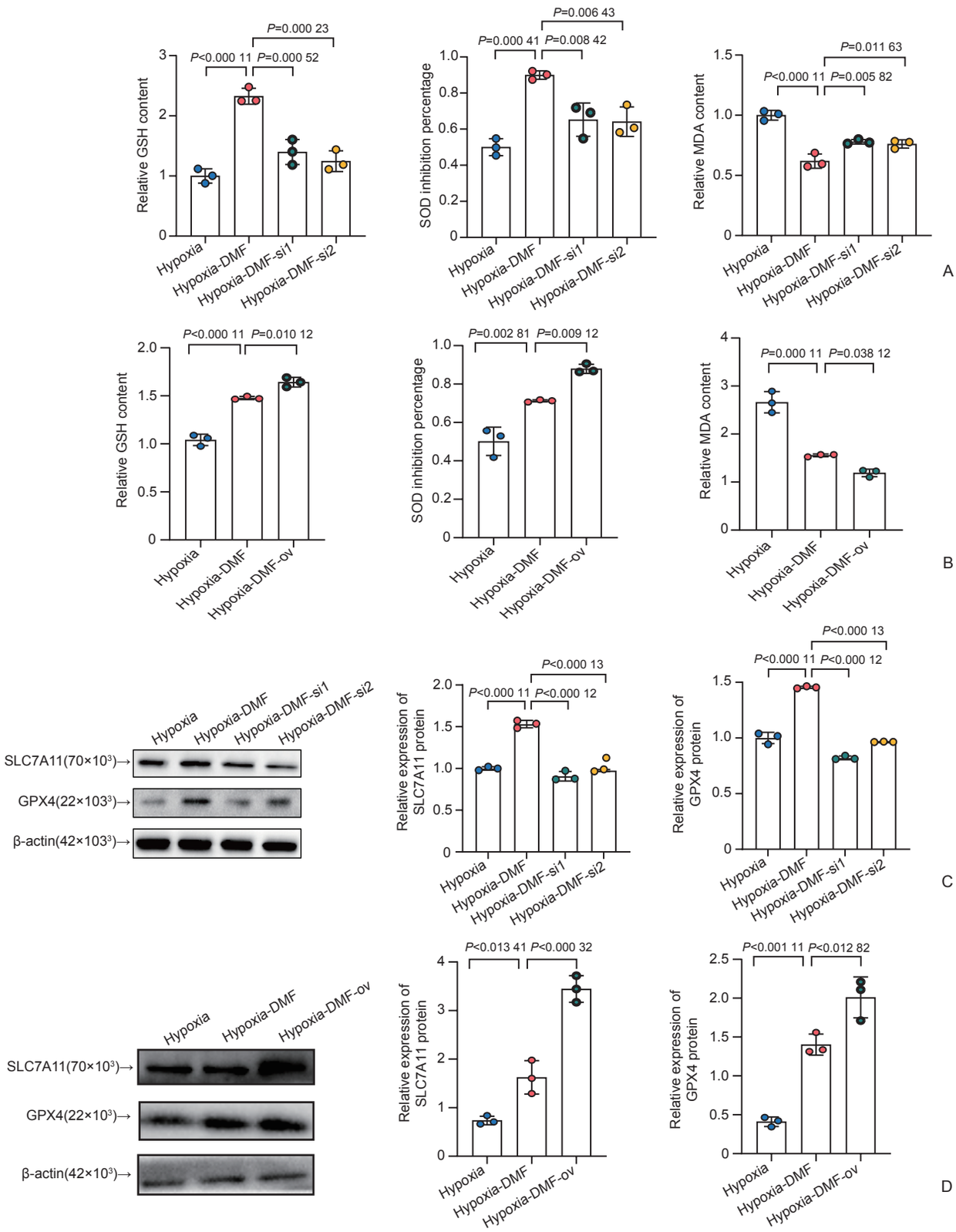


A~C: Hierarchical clustering (A), Volcano plot (B) and results of KEGG pathway enrichment analysis (C) of differentially expressed proteins between normoxic control and hypoxia-induced injury groups in BEAS-2B cells; D: Venn diagram showing overlapping proteins between differentially expressed proteins in BEAS-2B cells and ferroptosis-related proteins from the FerrDb V2 database (https://ferrdb.org); E: Expression levels of the 9 overlapping proteins in the proteomics data of BEAS-2B cells from control group and hypoxia group

图 5 高原低氧肺损伤 BEAS-2B 细胞蛋白质组学分析

Figure 5 Proteomic analysis of BEAS-2B cells in high-altitude hypoxia-induced lung injury

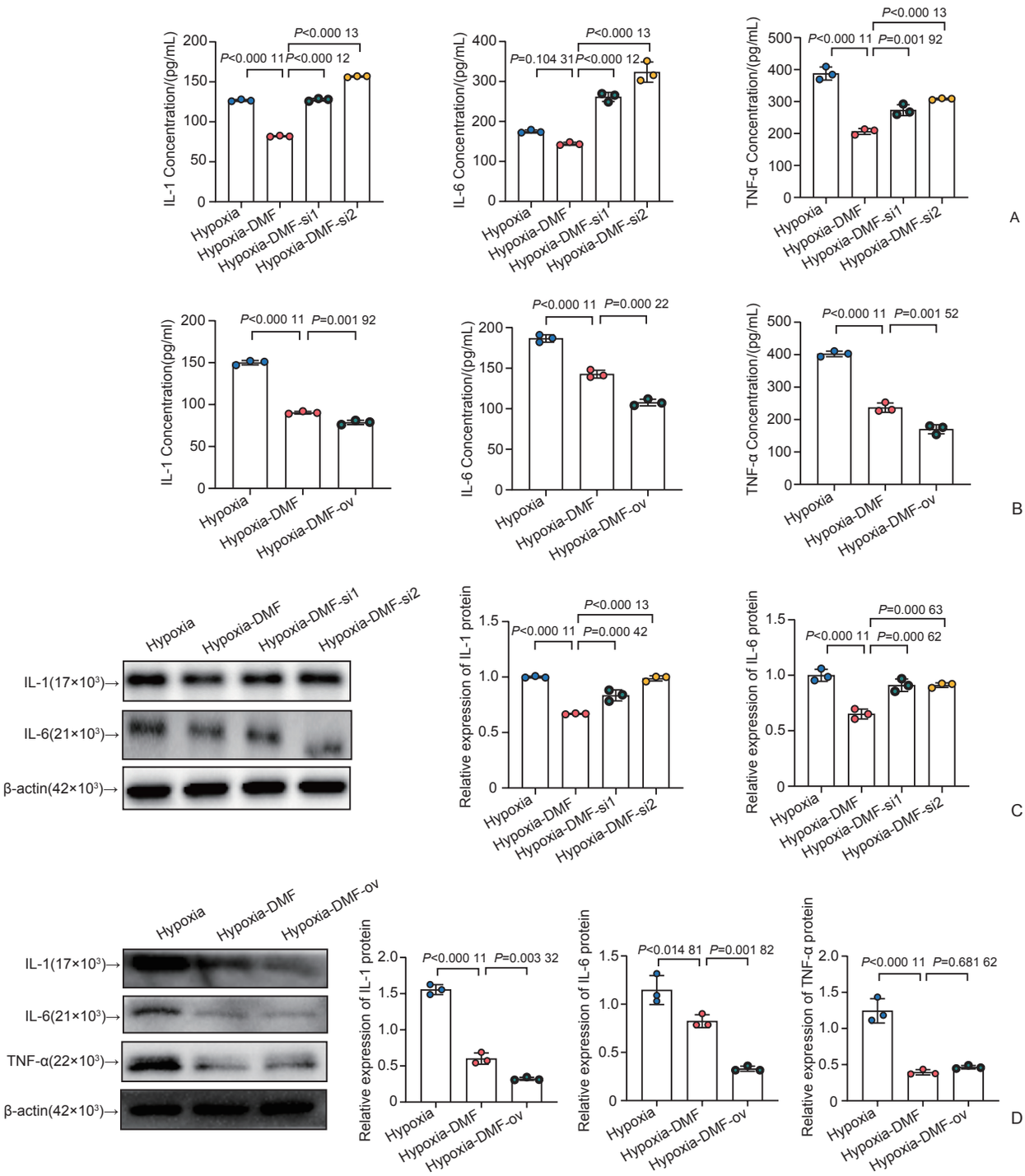
SLC7A11 蛋白质的稳定性和适当膜位。为了探寻 NRF2 是否是通过调控 SLC7A11 进而调控低氧肺损伤中 BEAS-2B 细胞铁死亡,以此减轻巨噬细胞炎症反应,本研究在 DMF 处理基础上,分别使用两种小干扰 RNA 及过表达质粒对 BEAS-2B 细胞中 SLC7A11 进行敲低和过表达。在给予 BEAS-2B 细胞 DMF 并敲低 *SLC7A11* 基因的表达后,相较于 DMF 预防组,DMF 预防敲低组 1 和 DMF 预防敲低组 2 巨噬细胞上清液中炎症因子(IL-1、IL-6、TNF-α)分泌显著回升(P<0.05,图 7A),巨噬细胞 IL-1 和 IL-6 在蛋白水平显著升高(P<0.05,图 7C);相反,在过表达 *SLC7A11* 后,相较于 DMF 预防组,DMF 预防过表达组巨噬细胞上清液中炎症因子(IL-1、IL-6、TNF-α)分泌显著降低(P<0.05,图 7B),巨噬细胞 IL-1、IL-6 和 TNF-α 在蛋白质水平上显著降低(P<0.05,图 7D)。低氧可促进巨噬细胞炎症反应,DMF 通过激活 NRF2 可抑制该反应,而 *SLC7A11* 敲低可部分逆转 DMF 的抗炎作用,SLC7A11 过表达则显著增强其效果,提示 DMF 通过 NRF2/SLC7A11 轴缓解巨噬细胞介导的炎症反应。综上结果表明,DMF 通过 NRF2/SLC7A11 轴缓解 BEAS-2B 细胞铁死亡,进而改善巨噬细胞的炎症反应,减轻高原低氧 ALI。



A, B: Effects of NRF2 upregulation+SLC7A11 knockdown (A) and NRF2 upregulation+SLC7A11 overexpression (B) on GSH content, SOD activity, and MDA content in BEAS-2B cells under hypoxic injury conditions; C, D: Protein expression of ferroptosis-related molecules in BEAS-2B cells after NRF2 upregulation+SLC7A11 knockdown (C) and NRF2 upregulation+SLC7A11 overexpression (D) under hypoxic injury conditions

图6 DMF通过NRF2/SLC7A11轴抑制低氧诱导的BEAS-2B细胞铁死亡的发生(n=5,$\bar{x}\pm s$)

Figure 6 DMF suppresses hypoxia-induced ferroptosis in BEAS-2B cells *via* the NRF2/SLC7A11 axis (n=5, $\bar{x}\pm s$)



A, B: Effects of NRF2 upregulation+SLC7A11 knockdown (A) and NRF2 upregulation+SLC7A11 overexpression (B) on the secretion of IL-1, IL-6 and TNF-α in macrophages; C, D: Protein levels of IL-1, IL-6 and TNF-α in macrophages after NRF2 upregulation+SLC7A11 knockdown (C) and NRF2 upregulation+SLC7A11 overexpression (D)

图7 DMF通过NRF2/SLC7A11轴抑制巨噬细胞炎症反应(n=5,$\bar{x}\pm s$)

Figure 7 DMF suppresses macrophage-mediated inflammatory response *via* the NRF2/SLC7A11 axis(n=5,$\bar{x}\pm s$)

3 讨论

高原地区特有的低压、低氧等严酷环境因素常导致呼吸与神经系统功能障碍,引发高原头痛、中枢性睡眠呼吸暂停综合征、高原脑水肿(high altitude cerebral edema, HACE)、高原肺水肿(high altitude pulmonary edema, HAPE)等病症[2]。在诸多高原诱发疾病的环境诱因中,低氧的作用最为关键,可诱发严

重的肺组织损伤。当前,高原低氧肺损伤的防治对策较多,药物干预是主要手段之一,但这些方法主要针对临床症状而非潜在病理机制。这使得深入阐明高原低氧肺损伤的分子机制,开发新型干预手段、减轻高原低氧环境对人体的负面效应至关重要。细胞死亡是急性肺损伤的重要病理环节之一,铁死亡作为铁依赖性的程序性细胞死亡特定类型,与神经退行性疾病、炎症反应及缺血-再灌注损伤等多种疾病病理机制显著相关[28-29],然而,在高原低氧所致肺损伤中,铁死亡的作用机制尚未得到充分研究。本课题组既往研究已经表明铁死亡在高原低氧肺损伤中发挥重要调控作用,但铁死亡调控高原低氧肺损伤的具体机制仍然不清楚。基于上述背景,本研究在动物和细胞水平分别探讨了DMF上调NRF2对低氧诱导的急性肺损伤的保护效应,随后对高原低氧肺损伤BEAS-2B细胞进行蛋白组学分析以确定SLC7A11为NRF2的关键下游调控分子,在细胞模型中详细探讨了DMF通过NRF2/SLC7A11轴抑制上皮细胞铁死亡并缓解巨噬细胞炎症反应从而对高原低氧肺损伤发挥保护作用,这为高原低氧肺损伤的防治策略提供新思路。

3.1 DMF上调NRF2抑制高原低氧诱导的肺上皮细胞铁死亡从而减轻巨噬细胞炎症反应

铁死亡的特征性表现涉及铁离子蓄积与脂质过氧化反应,且众多参与抗氧化系统、铁稳态及脂质代谢的相关基因均参与调控该过程。NRF2作为关键转录因子,通过调控拮抗氧化与亲电子应激的基因表达,在维持细胞抗氧化能力中发挥核心作用并调控多种疾病铁死亡的发生[30-32]。NRF2可以影响胶质瘤细胞对铁死亡诱导剂Erastin和RSL3的敏感性,沉默NRF2能够损害咽鳞状细胞癌中癌细胞的生长,提高细胞ROS水平[33]。NRF2还可通过抑制铁死亡来缓解海水溺水所致的肺损伤。NRF2抗氧化反应可通过减弱铁死亡进而改善高原肺水肿[34]。但关于NRF2在高原低氧ALI铁死亡中的作用尚缺乏研究报道,为了探究NRF2在高原低氧肺损伤中的作用机制,我们随后构建高原低氧肺损伤大鼠模型和BEA-2B细胞-巨噬细胞共培养模型,给予NRF2激动剂DMF,观察到上调NRF2表达可改善高原低氧诱导的脂质过氧化水平,抑制铁死亡的发生;除此之外,DMF上调BEAS-2B细胞NRF2表达升高的同时可降低高原低氧肺损伤中巨噬细胞炎症因子的表达和分泌,提示DMF可通过NRF2依赖的铁死亡途径对高原低氧ALI发挥保护作用。

3.2 DMF通过NRF2/SLC7A11轴抑制高原低氧肺损伤铁死亡进而减轻炎症反应

铁死亡级联反应中的许多分子是NRF2的靶点,有研究报道,花青素可以通过NRF2/HO-1/KEAP-1途径调节铁死亡,从而减轻LPS诱导的RAW264.7细胞的炎症[35]。激活NRF2/TXNRD1轴能够减轻蛛网膜下腔出血后海马神经元的铁死亡和炎症反应[36]。调节NRF2/HO-1轴可减轻铁死亡,缓解炎症反应,从而抑制对乙酰氨基酚诱导的肝损伤[37]。但NRF2调控低氧ALI中铁死亡进而影响ALI炎症反应的分子机制尚不明确。在铁死亡发生机制中,胱氨酸/谷氨酸反转运系统Xc^-是广泛存在于磷脂双分子层的氨基酸反转运蛋白,通过1∶1的数量比例将细胞外胱氨酸输送到细胞内,将细胞内谷氨酸输送到细胞外,从而在调控细胞内和细胞外氨基酸平衡方面发挥重要作用,Xc^-系统由两个亚基组成,分别是SLC7A11和SLC3A2,SLC7A11负责胱氨酸和谷氨酸在质膜上的运输,SLC3A2则作为SLC7A11伴侣蛋白[38]。既往研究发现NRF2/SLC7A11在多种疾病中调控铁死亡,但关于低氧下BEAS-2B细胞NRF2是否能通过SLC7A11调控铁死亡进而影响巨噬细胞介导的炎症反应目前尚未有研究报道。因此本研究通过小干扰RNA沉默BEAS-2B细胞的*SLC7A11*基因后,观察到前期通过DMF上调NRF2表达而改善的BEAS-2B细胞铁死亡现象和巨噬细胞介导的炎症反应均被SLC7A11表达降低所逆转,提示NRF2/SLC7A11轴在低氧ALI中通过调控铁死亡进而调控炎症反应。与常氧对照组相比,低氧肺损伤组GSH、GPX4水平下降,ROS水平升高,由此我们推测在体外高原低氧肺损伤模型中,持续的低氧共培养使BEAS-2B细胞NRF2表达降低,调控SLC7A11表达下降,影响胱氨酸转运,进而影响下游GSH合成,使以GSH为辅因子的GPX4活性降低,脂质过氧化物持续积累,最终导致BEAS-2B细胞发生铁死亡,进而影响低氧ALI的炎症反应。

3.3 高原低氧肺损伤中BEAS-2B细胞铁死亡会促进巨噬细胞极化从而加剧炎症反应

铁死亡过程中,多种炎症相关基因的表达产物水平升高,尤以TNF-α、IL-6、CCL2、CCL7和CCL12为主。这些因子可刺激巨噬细胞的募集与趋化,进而增强免疫应答[18,39-41]。巨噬细胞是免疫系统的核心组成,主要表现为M1型和M2型两种极化表型,具有促炎特性的M1型巨噬细胞可清除病原体并诱导免疫应答,其中高表达诱导型一氧化氮合酶(iNOS)的M1型巨噬细

胞表现出显著的抗肿瘤活性[42],而M2型巨噬细胞则发挥抗炎功能[43]。铁死亡细胞释放的HMGB1能与巨噬细胞表面受体AGER结合,调控巨噬细胞的炎症反应,该机制可能与肾炎、脓毒症、胰腺炎及各类缺血再灌注组织损伤中炎症反应的发生与维持相关[44]。铁死亡的肺上皮细胞通过HMGB1与AGER的相互作用上调巨噬细胞TNF-α[44],作为关键促炎因子,升高的TNF-α不仅调控促炎细胞因子产生,还增强脂质信号转导调节因子(如血小板活化因子和前列腺素)的表达,从而推动多种炎症性疾病的发展[45]。此外,铁死亡细胞分泌的CCL2与CCL7通过结合巨噬细胞表面CCR2受体,介导巨噬细胞的募集、趋化及极化过程[46]。在特定条件下,巨噬细胞会增强铁死亡敏感性并加速疾病恶化。WU等[47-48]研究表明,当巨噬细胞暴露于缺氧/复氧环境并与肝细胞共培养时,其胞外诱捕网的增多可促进肝细胞铁死亡,从而加剧肝脏缺血/再灌注损伤后的缺血性肝损伤。在心肌梗死模型中,铁死亡通过降低心肌细胞外泌体miR-106b-3p含量激活Wnt/β-连环蛋白通路,最终诱导巨噬细胞极化为M1型表型[49]。与前期研究结果一致,在高原低氧肺损伤中,大鼠肺组织铁死亡发生的同时,检测到支气管灌洗液中M1型巨噬细胞比例增加,而M2型巨噬细胞比例降低;此外在BEAS-2B细胞-巨噬细胞共培养的ALI细胞模型中,BEAS-2B细胞发生铁死亡,M1型巨噬细胞表面标志物表达和炎症因子分泌也均显著升高,而给予DMF抑制BEAS-2B细胞铁死亡后巨噬细胞M1型比例和炎症因子水平显著降低。这表明在高原低氧肺损伤中BEAS-2B细胞铁死亡可调控巨噬细胞极化从而加剧炎症反应。但在高原低氧ALI中,发生铁死亡的BEAS-2B细胞通过何种蛋白或通路调控巨噬细胞的极化状态,有待进一步研究。

综上,本研究基于课题组前期工作锁定铁死亡在高原低氧肺损伤的发生发展中发挥重要作用。随后在本研究中利用体内体外实验中探索在低氧环境下NRF2对铁死亡和炎症反应的调控机制。实验结果显示,NRF2分子的表达受低氧调控而下降,进而影响其下游靶分子SLC7A11使其表达降低。作为铁死亡关键抑制蛋白和胱氨酸谷氨酸反转运系统的功能蛋白,SLC7A11的降低引起了下游重要还原剂GSH水平降低,以及发挥抗氧化作用的蛋白酶GPX4水平和SOD活性下降,随之引起ROS的积累,细胞抗氧化能力下降,发生铁死亡,进而导致在低氧环境下巨噬细胞炎症因子表达和分泌增多,而在低氧环境下炎症反应加重的同时铁死亡也相应加重,形成恶性循环,最终加重低氧所致的急性肺损伤。这一研究发现为高原低氧肺损伤的分子机制研究奠定了一定的基础,也为其防治策略提供新思路。

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