论著·基础医学

蛋白磷酸酶2A促进线粒体自噬减轻果糖诱导的M2型巨噬细胞线粒体氧化损伤

李晓蔓[1],蓝 利[1,2],龙以瑾[1],李慧莲[1],王名鸿[3],王新航[1,2],李习艺[3],唐 深[1,2] 广西医科大学:[1]基础医学院免疫学教研室,[2]广西高校基础医学研究重点实验室,[3]公共卫生学院营养与食品卫生学系,广西南宁

[摘要] **目的** 研究果糖暴露对M2型巨噬细胞线粒体氧化损伤的影响,并采用蛋白磷酸酶甲酯化酶-1(protein phosphatase methylesterase-1,PPME-1)抑制剂ABL127特异性激活蛋白磷酸酶2A(protein phosphatase 2A,PP2A)以阐明其调控作用。**方法** ①以永生化小鼠骨髓来源巨噬细胞系(Ana-1)为研究对象,设置M0组(常规培养)、M2组(20.00 ng/mL IL-4处理24 h)及M2+梯度果糖处理组(20 ng/mL IL-4联合0.04、0.20、1.00、5.00 mmol/L果糖处理24 h)。采用CCK-8法检测细胞活性,荧光探针法检测线粒体数量、细胞总活性氧(reactive oxygen species,ROS)、线粒体源ROS及线粒体膜电位水平,Western blot法检测PP2Ac总蛋白(total-PP2Ac)及去甲基化PP2Ac(demethylated-PP2Ac)蛋白表达水平。②设置M0组、M2组、M2+Fru组(20.00 ng/mL IL-4+5.00 mmol/L果糖处理24 h)及M2+Fru+ABL127组(20.00 ng/mL IL-4+5.00 mmol/L果糖+1.00 μmol/L ABL127处理24 h)探究PP2A干预机制。荧光探针法检测线粒体与溶酶体数量、ROS及线粒体膜电位水平,Western blot法检测线粒体自噬相关蛋白PTEN诱导激酶1(PTEN induced putative kinase 1,PINK1)、自噬底物P62(sequestosome 1,SQSTM1/P62)、微管相关轻链蛋白3(microtubule-associated protein light chain 3,LC3)及电压依赖性阴离子通道(voltage-dependent anion channel,VDAC)表达,RT-qPCR检测M2型巨噬细胞表型标志物炎症区域分子1(found in inflammatory zone 1,Fizz1)、精氨酸酶1(arginase-1,Arg-1)及转化生长因子-β(transforming growth factor-β,TGF-β)的mRNA表达。**结果** ①与M2组相比,0.04~5.00 mmol/L梯度果糖处理组在不影响细胞活性前提下呈剂量依赖性诱导M2型巨噬细胞总ROS水平升高($P<0.05$),果糖浓度达5.00 mmol/L时线粒体源ROS及线粒体数量增加($P<0.05$),线粒体膜电位水平下降($P<0.05$),同时Total-PP2Ac未见改变,而Demethylated-PP2Ac蛋白表达显著上调($P<0.05$)。②与M2+Fru组相比,M2+Fru+ABL127组线粒体数量减少、溶酶体数量增加($P<0.01$),线粒体自噬相关蛋白PINK1、LC3Ⅱ及VDAC表达均上调($P<0.05$),自噬底物P62表达下调($P<0.05$),总体及线粒体源ROS水平降低、线粒体膜电位上升($P<0.01$);与M2组相比,M2+Fru组标志物*Fizz1*、*Arg-1*及*TGF-β*的mRNA表达下降($P<0.05$),ABL127干预后上述标志物表达升高($P<0.05$)。**结论** 果糖诱导M2型巨噬细胞PP2Ac去甲基化造成线粒体氧化损伤,激活PP2A显著促进线粒体自噬以逆转该损伤。

[关键词] 果糖;巨噬细胞;线粒体自噬;氧化应激;蛋白磷酸酶2A

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[通信作者] 唐 深,E-mail:958714333@qq.com

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Protein phosphatase 2A promotes mitophagy to alleviate fructose-induced mitochondrial oxidative damage in M2-type macrophages

LI Xiaoman[1], LAN Li[1,2], LONG Yijin[1], LI Huilian[1], WANG Minghong[3], WANG Xinhan[1,2], LI Xiyi[3], TANG Shen[1,2]

[1]Department of Immunology, School of Basic Medical Sciences, [2]Key Laboratory of Basic Medical Sciences of Guangxi Higher Education Institutions, [3]Department of Nutrition and Food Hygiene, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

[**Abstract**] Objective To investigate the effects of fructose exposure on mitochondrial oxidative damage in M2-type macrophages and elucidate the regulatory role of protein phosphatase 2A (PP2A) in the process using its specific activator ABL127, an inhibitor of protein phosphatase methylesterase-1 (PPME-1). Methods ① Immortalized mouse bone marrow-derived macrophages Ana-1 were subjected and grouped into M0 (conventional culture), M2 (treated with 20 ng/mL IL-4 for 24 h), and M2+Fru groups (IL-4 plus 0.04, 0.20, 1.00, or 5.00 mmol/L fructose). Cell viability was assessed with CCK-8 assay. Number of mitochondria, total and mitochondrial levels of reactive oxygen species (ROS), and mitochondrial membrane potential (ΔΨM) were measured using fluorescent probes. Total and demethylated PP2Ac protein levels were detected by Western blotting. ② Ana-1 cells were also divided into M0, M2, M2+Fru (20 ng/mL IL-4+5.00 mmol/L fructose, 24 h), and M2+Fru+ABL127 (20 ng/mL IL-4+5.00 mmol/L fructose+1.00 μmol/L ABL127, 24 h) to investigate PP2A-mediated mechanisms. Numbers of mitochondria and lysosomes, ROS level, and ΔΨM were detected *via* fluorescence assays. Expression of mitophagy-related proteins, PTEN induced putative kinase 1 (PINK1), P62, microtubule-associated protein light chain 3 (LC3), and voltage-dependent anion channel (VDAC) was evaluated by Western blotting, and the mRNA levels of M2 markers, found in inflammatory zone 1 (*Fizz1*), arginase-1 (*Arg-1*), and *TGFβ* were measured using RT-qPCR. Results ①Compared with the M2 group, fructose treatment at a concentration ranging from 0.04 to 5.00 mmol/L showed no effect on cell viability in M2 macrophages, but increased total ROS level in a dose-dependent manner ($P<0.05$). Fructose of 5.00 mmol/L resulted in significantly elevated mitochondrial ROS and mitochondrial quantity ($P<0.05$), reduced ΔΨM ($P<0.05$), up-regulated demethylated-PP2Ac ($P<0.05$), and no changed total-PP2Ac protein level. ② Compared with the M2 + Fru group, the addition of ABL127 led to decreased number of mitochondria but increased number of lysosomes ($P<0.01$), up-regulation of PINK1, LC3 Ⅱ and VDAC proteins, down-regulation of P62 ($P<0.05$), reduced total and mitochondrial ROS levels, and enhanced ΔΨM ($P<0.01$). The mRNA expression of *Fizz1*, *Arg-1*, and *TGF-β* was notably decreased in the M2+Fru group than the M2 group ($P<0.05$), and the levels were rescued by ABL127 treatment ($P<0.05$). Conclusion Fructose induces PP2Ac demethylation and then mitochondrial oxidative damage in M2-type macrophages. PP2A activation promotes mitophagy and reverses fructose-induced damage.

[**Key words**] fructose; macrophages; mitophagy; oxidative stress; protein phosphatase 2A

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果糖在现代饮食中摄入量持续攀升。相较于葡萄糖,生物体内的果糖含量较低,其循环果糖浓度通常为0.04~0.20 mmol/L,短期摄入大量果糖可使其在肝脏门静脉中的浓度升高至1.00 mmol/L;而在急性髓性白血病的骨髓微环境中果糖摄取浓度甚至可达5.00 mmol/L[1]。在机体中,果糖代谢通过规避磷酸果糖激酶-1的限速作用,促使代谢中间产物大量积累[2],并引发活性氧(reactive oxygen species,ROS)蓄积,从而损害线粒体呼吸功能及膜电位,最终导致线粒体功能障碍[3]。巨噬细胞作为固有免疫系统的核心成员,

其功能高度依赖于线粒体稳态与代谢[4]。在不同微环境刺激下,巨噬细胞可极化为以糖酵解为主的促炎性M1型或依赖氧化磷酸化的修复性M2型表型[5]。过量ROS蓄积会破坏线粒体稳态,严重损害依赖于线粒体能量代谢的M2型巨噬细胞。这不仅削弱了其抗炎与组织修复能力,还可能驱动其向促炎表型转化[6]。然而,目前对于果糖如何通过诱导ROS生成及线粒体损伤来影响M2型巨噬细胞的功能的具体机制,尚不完全明确。

蛋白磷酸酶2A(protein phosphatase 2A,PP2A)是一种保守的丝氨酸/苏氨酸蛋白磷酸酶,其活性依赖于催化亚基PP2Ac的甲基化修饰[7],其中亮氨酸羧基甲基转移酶-1和蛋白磷酸酶甲酯化酶-1(protein phosphatase methylesterase-1,PPME-1)分别调控其甲基化与去甲基化状态[8]。选择性共价PPME-1抑制剂ABL127,通过特异性抑制PPME-1间接增强PP2Ac甲基化,从而激活PP2A[9]。PP2A活性对于维持线粒体稳态具有重要作用,其通过去磷酸化修饰抗氧化相关分子[10]及自噬信号通路蛋白[11]等途径广泛参与调控细胞氧化应激水平和线粒体自噬进程。前期研究指出PP2A在H_2O_2诱导的氧化损伤中起保护作用[12],但其在果糖干预的M2型巨噬细胞调控导线粒体氧化损伤与线粒体自噬的机制尚未明确。本研究拟利用果糖处理的M2型巨噬细胞模型,探究果糖对线粒体氧化损伤的影响,并阐明ABL127调控的PP2A活性在果糖诱导的线粒体氧化应激及线粒体自噬中的作用。

1 材料与方法

1.1 实验材料与试剂

永生化小鼠骨髓来源巨噬细胞系(Ana-1)购自中国科学院典型培养物保藏委员会细胞库;RPMI 1640培养基购自美国Gibco公司;D-果糖、细胞增殖及毒性检测试剂盒(CCK-8法)购自大连美仑生物技术有限公司;小鼠源白细胞介素4(interferon-4,IL-4)购自美国MedChemExpress公司;ABL127购自美国Sigma公司;PTEN诱导激酶1(PTEN induced putative kinase 1,PINK1)抗体、PP2Ac总蛋白(total-PP2Ac)及去甲基化PP2Ac(demethylated-PP2Ac)抗体购自美国Santa Cruz公司;兔抗微管相关1轻链3(microtubule-associated protein 1 light chain 3,LC3)抗体、自噬选择性底物P62(sequestosome 1,SQSTM1/P62)抗体、电压依赖性阴离子通道(voltage-dependent anion channel,VDAC)抗体购自美国Cell Signaling Technology公司;辣根过氧化物酶(horseradish peroxidase,HRP)标记山羊抗小鼠或抗兔IgG、EVOS FL Auto全自动细胞成像系统、iBright CL750 ECL成像系统购自美国Thermo Fisher Scientific公司;BCA蛋白质定量试剂盒、M-MLV逆转录试剂盒购自日本TaKaRa公司;SYBR®Green Master购自苏州近岸蛋白生物有限公司;RIPA裂解液、β-Tubulin抗体、2′,7′-二氯二氢荧光素二乙酸酯探针(DCFH-DA)、线粒体超氧化物探针(mitoSOX-Red)、线粒体绿色荧光探针(Mito-Tracker-Green)、溶酶体红色荧光探针(Lyso-Tracker-Red)及线粒体膜电位检测探针(JC-1)购自上海碧云天生物技术有限公司;TRIzol裂解液购自北京天根生化科技有限公司;SparK 10M多功能酶标仪购自瑞士Tecan公司;垂直电泳仪购自美国Bio-Rad公司;LightCycler® 480 Ⅱ购自德国Roche公司。

1.2 实验方法

1.2.1 实验细胞培养及分组 Ana-1细胞在添加10%胎牛血清及双抗(青霉素100 IU/mL、链霉素100 μg/mL)的RPMI 1640培养基中进行体外培养,并维持于37 ℃恒温、5% CO_2环境下生长。无菌水配置果糖溶液待用。取处于对数生长期的Ana-1细胞按6×10^5或2×10^4个/孔接种于6孔板或96孔板,分为以下各处理组:实验一,M0组(常规培养)、M2组(20.00 ng/mL IL-4处理24 h)、M2+梯度果糖处理组(20.00 ng/mL IL-4联合0.04、0.20、1.00及5.00 mmol/L果糖处理24 h);实验二,M0组、M2组、M2+Fru组(20.00 ng/mL IL-4+5.00 mmol/L果糖处理24 h)及M2+Fru+ABL127组(20.00 ng/mL IL-4+5.00 mmol/L果糖+1.00 μmol/L ABL127处理24 h)。

1.2.2 CCK-8法检测细胞增殖活性 Ana-1细胞接种于96孔板,经1.2.1实验处理后,采用换液方式向各处理组加入含有10% CCK-8试剂的RPMI 1640培养基100 μL,于37 ℃下避光孵育2 h。使用多功能酶标仪检测各孔吸光度[OD(450)]值,以评估细胞活性。

1.2.3 DCFH-DA及mitoSOX-Red探针检测细胞总体及线粒体源ROS水平 Ana-1细胞接种于96孔板,经1.2.1实验处理后,使用1×PBS将DCFH-DA和mitoSOX Red探针母液按1∶1 000体积稀释,配置成复合染料工作液,每孔加入100 μL染色工作液,避光孵育30 min,用1×PBS洗涤2次,20 min内于荧光酶标仪根据396 nm激发波长、610 nm发射波长检测mitoSOX-Red红色荧光强度;根据488 nm激发波长、525 nm发射波长测定DCFH-DA绿色荧光信号强度。

1.2.4 JC-1探针红绿荧光比值评估线粒体膜电位变化

Ana-1细胞接种于96孔板,经1.2.1实验处理后,按照50 μL JC-1(200×)、8 mL纯水、2 mL JC-1染色缓冲液(5×)比例配置JC-1染色工作液,每孔加入100 μL染色工作液,避光孵育30 min。在高膜电位状态下,JC-1探针在线粒体基质内聚集成红色荧光聚合体;当膜电位降低时,探针以单体形式存在并发出绿色荧光。荧光酶标仪分别根据490 nm激发波长、530 nm发射波长测定绿色荧光信号强度,或525 nm激发波长、590 nm发射波长测定红色荧光信号强度。并计算红色/绿色荧光信号强度衡量线粒体去极化程度。EVOS FL Auto全自动细胞成像系统采集JC1探针聚合体及单体荧光图像。

1.2.5 Mito-Tracker-Green及Lyso-Tracker-Red探针检测细胞线粒体及溶酶体数量 Ana-1细胞接种于96孔板,经1.2.1实验处理后,无血清RPMI 1640培养基参照说明书稀释Mito-Tracker-Green及Lyso-Tracker-Red探针,每孔加入100 μL染色工作液避光孵育30 min,1×PBS洗涤2次后于荧光酶标仪根据激发波长577 nm、发射波长590 nm采集溶酶体荧光信号强度,及激发波长490 nm、发射波长516 nm采集线粒体荧光信号强度,并采用EVOS FL Auto全自动细胞成像系统采集细胞内线粒体和溶酶体荧光图像。

1.2.6 Western blot法检测细胞PP2Ac去甲基化及线粒体自噬相关蛋白表达 Ana-1细胞接种于6孔板,经1.2.1实验处理后,加入RIPA裂解液提取蛋白,采用BCA比色法检测蛋白质浓度。SDS-PAGE电泳实施蛋白分离,电转至PVDF膜后,采用5%脱脂乳-TBST封闭体系室温孵育2 h。TBST缓冲液漂洗30 min,采用如下稀释比例的一抗4 ℃孵育过夜:β-Tubulin(1∶4 000)、Total-PP2Ac(1∶4 000)、Demethylated-PP2Ac(1∶2 000)、PINK1(1∶1 000)、P62(1∶1 000)、VDAC(1∶2 000)、LC3(1∶1 000)。回收一抗,TBST缓冲液漂洗30 min后按照1∶2 000加入HRP标记IgG抗体,常温孵育2 h。采用ECL法于iBright CL750 ECL成像系统进行曝光显影。使用Image J软件对蛋白条带进行灰度值分析,目的蛋白表达水平以内参蛋白β-Tubulin进行归一化处理后进行统计分析。

1.2.7 RT-qPCR检测M2巨噬细胞标志物*Fizz1*、*Arg-1*及*TGF-β*基因表达 Ana-1细胞接种于6孔板,经1.2.1实验二处理后,TRNzol裂解液提取各处理组细胞RNA,参照TaKaRa公司M-MLV逆转录试剂说明书逆转录为cDNA。构建包含SYBR Green Master 10 μL、PCR正向引物(10.00 μmol/L)1 μL、PCR反向引物(10.00 μmol/L)1 μL、无RNA酶水6 μL及cDNA 200 ng的PCR反应体系。以β-actin为内参基因,采用$2^{-\Delta\Delta Ct}$计算各处理组炎症区域分子1(found in inflammatory zone 1,Fizz1)、精氨酸酶1(Arginase-1,Arg-1)及转化生长因子-β(transforming growth factor-β,TGF-β)基因相对表达量。引物序列见表1。

1.3 统计学分析

采用SPSS 23.0进行统计学处理,统计图表使用GraphPad Prism 9软件绘制,结果以$\bar{x} \pm s$表示。符合正态分布及方差齐性检验的资料,多组间比较采用单因素ANOVA法,组间两两比较采用LSD-*t*法,方差不齐采用Games-Howell检验;资料不符合正态分布时使用非参数检验。所有实验独立重复3次及以上,$P<0.05$表示差异具有统计学意义。

2 结果

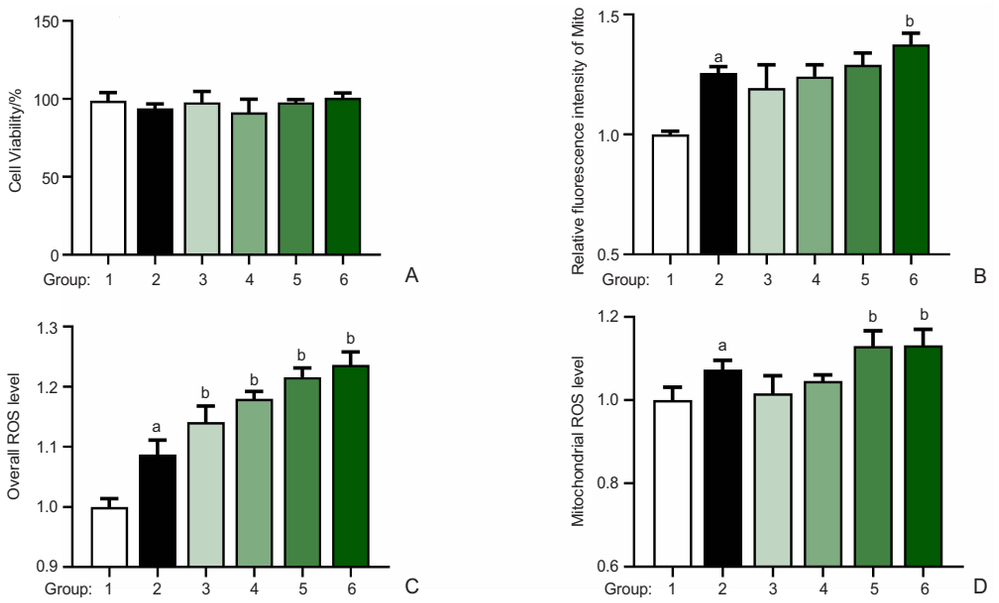
2.1 果糖促进M2型巨噬细胞ROS累积及线粒体生成

相较于M0组,M2组线粒体数量增多、细胞总ROS水平及线粒体源ROS水平升高($P<0.01$,图1B~D);与M2组相比,0.04~5.00 mmol/L果糖处理对巨噬细胞活性无显著影响,果糖浓度达到5.00 mmol/L时可见线粒体数量增加($P<0.05$,图1B)。此外,细胞总ROS水平在0.04~5.00 mmol/L果糖范围内呈剂量依赖性升高($P<0.05$,图1C),而线粒体源ROS水平在果糖浓度≥1.00 mmol/L时增加($P<0.05$,图1D)。以上结果提示,果糖可能通过增强线粒体生物合成而非促进细胞增殖,进而诱导细胞内ROS的累积。

表1 基因名称及引物序列(鼠,5′→3′)

Table 1 Gene names and primer sequences (Mice,5′→3′)

Gene	PCR Forward Primer	PCR Reverse Primer
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Fizz1	CCAATCCAGCTAACTATCCCTCC	ACCCAGTAGCAGTCATCCCA
Arg-1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
TGF-β	CTTCGACGTGACAGACGCT	GCAGGGGCAGTGTAAACTTATT



1: M0 group; 2: M2 group (20 ng/mL IL-4); 3: M2+0.04 mmol/L Fru group; 4: M2+0.20 mmol/L Fru group; 5: M2+1.00 mmol/L Fru group; 6: M2+5.00 mmol/L Fru group. ROS: reactive oxygen species; Mito: mitochondrial. a: $P<0.01$, in comparison with the M0 group; b: $P<0.05$, in comparison with the M2 group; A: Cell viability after gradient fructose treatment; B: Relative fluorescence intensity of mitochondria after gradient fructose treatment; C: Overall ROS level after gradient fructose treatment; D: Mitochondrial ROS level after gradient fructose treatment

图1 果糖对M2型巨噬细胞活性、线粒体数量及ROS水平的影响

Figure 1 Effects of fructose on the viability, mitochondrial quantity and ROS level of M2-type macrophages

2.2 果糖诱导M2型巨噬细胞线粒体膜电位下降

通过检测线粒体膜电位水平评估线粒体功能。实验结果表明,与M0组相比,M2组巨噬细胞线粒体膜电位无明显变化;与M2组相比,0.04~1.00 mmol/L果糖处理也未对该指标产生显著影响,当果糖浓度达到5.00 mmol/L时,镜下可见单体绿色荧光强度无明显变化,但聚合体红色荧光减弱,线粒体膜电位水平下降($P<0.05$,图2B)。该结果提示,5.00 mmol/L果糖微环境可能导致M2型巨噬细胞线粒体功能障碍。

2.3 果糖诱导M2型巨噬细胞PP2Ac去甲基化抑制PP2A活性

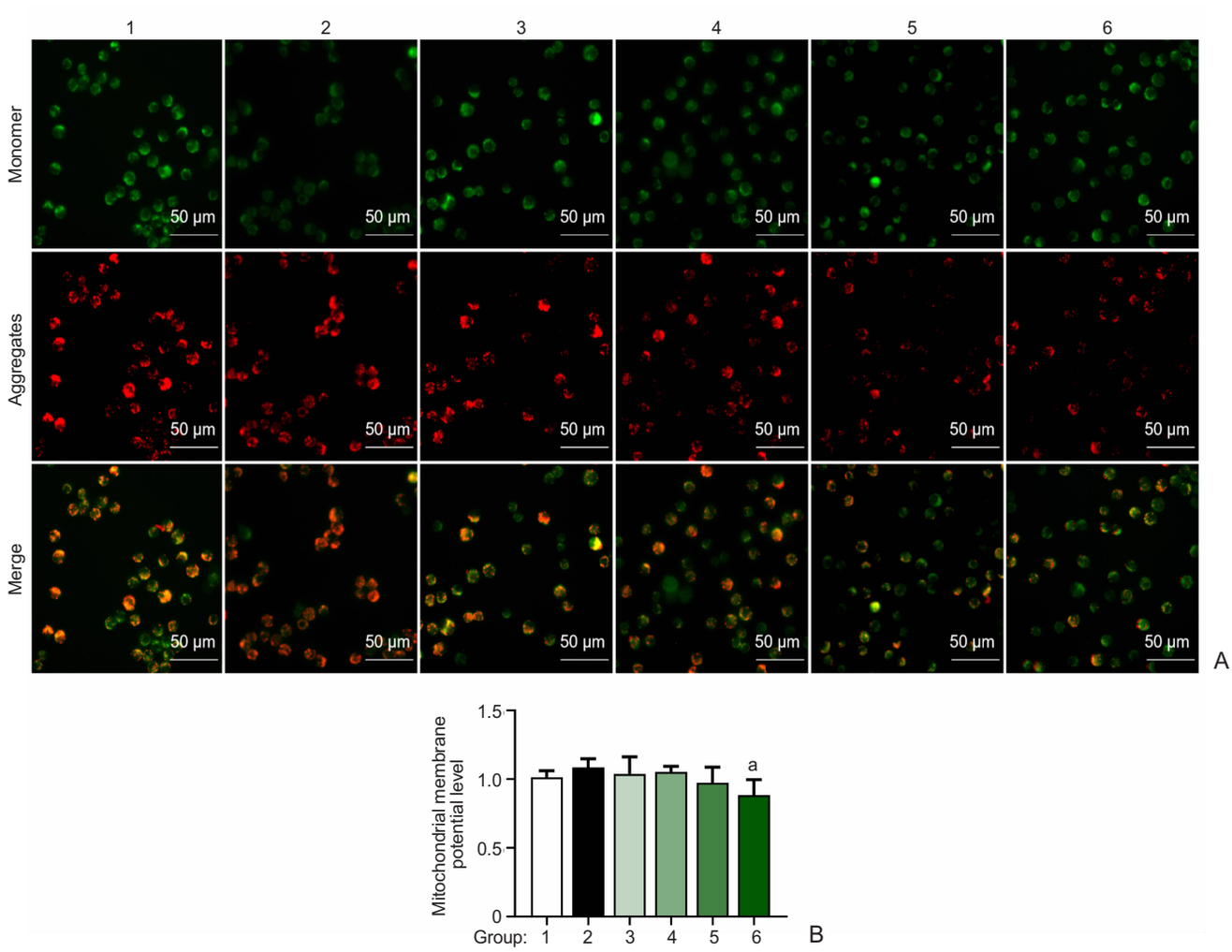
结果显示,与M2组相比,在梯度浓度果糖刺激下细胞中demethylated-PP2Ac的表达呈浓度依赖性上调,且在果糖浓度达到1.00 mmol/L和5.00 mmol/L时,该上调作用具有统计学显著性($P<0.05$,图3B);而total-PP2Ac表达水平未见显著改变。以上结果表明,果糖通过上调去甲基化PP2Ac表达,进而抑制M2型巨噬细胞内PP2A整体酶活性。

2.4 ABL127促进线粒体自噬减少果糖诱导的M2型巨噬细胞线粒体累积

结果显示,ABL127处理前后细胞活性未见明显改变(图4A)。与M2+Fru组相比,M2+Fru+ABL127组线粒体数量减少($P<0.01$,图4B),同时溶酶体数量增多($P<0.01$,图4C);Western blot结果表明,与M2组相比,M2+Fru组线粒体自噬相关蛋白PINK1、P62、VDAC及LC3Ⅱ表达未发生显著变化;与M2+Fru组相比,M2+Fru+ABL127组PINK1、VDAC及LC3Ⅱ蛋白表达均上调($P<0.05$),自噬底物P62表达下调($P<0.05$,图5)。上述结果提示,ABL127可能通过促进溶酶体生物合成及上调线粒体自噬蛋白表达,增强线粒体自噬活性,促进果糖诱导下过量线粒体的清除。

2.5 ABL127减少果糖诱导的M2型巨噬细胞ROS生成并恢复线粒体膜电位

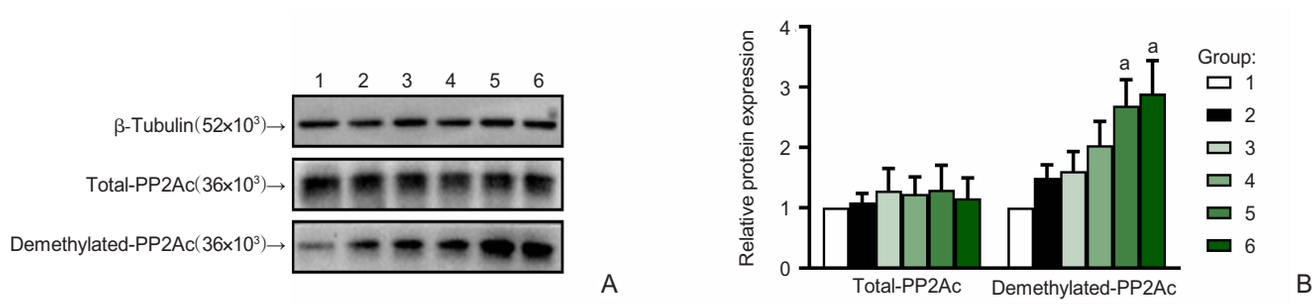
结果显示,与M2组相比,M2+Fru组细胞的ROS水平升高,线粒体膜电位下降($P<0.05$,图6);与M2+Fru组相比,M2+ Fru+ABL127组细胞总体ROS水平明显下降,线粒体源ROS下降趋势与总体ROS水平一致($P<0.01$,图6A、B),同时线粒体膜电位回升($P<0.01$,图6C)。上述结果提示,ABL127能够有效抑制细胞内总ROS及线粒体源ROS的生成,并修复线粒体膜电位水平。



1: M0 group; 2: M2 group (20 ng/mL IL-4); 3: M2+0.04 mmol/L Fru group; 4: M2+0.20 mmol/L Fru group; 5: M2+1.00 mmol/L Fru group; 6: M2+5.00 mmol/L Fru group. a: $P<0.05$, in comparison with the M2 group; A: Changes in fluorescence intensity of JC1 under a fluorescence microscope; B: Mitochondrial membrane potential level after gradient fructose treatment

图2 果糖对M2型巨噬细胞线粒体膜电位的影响

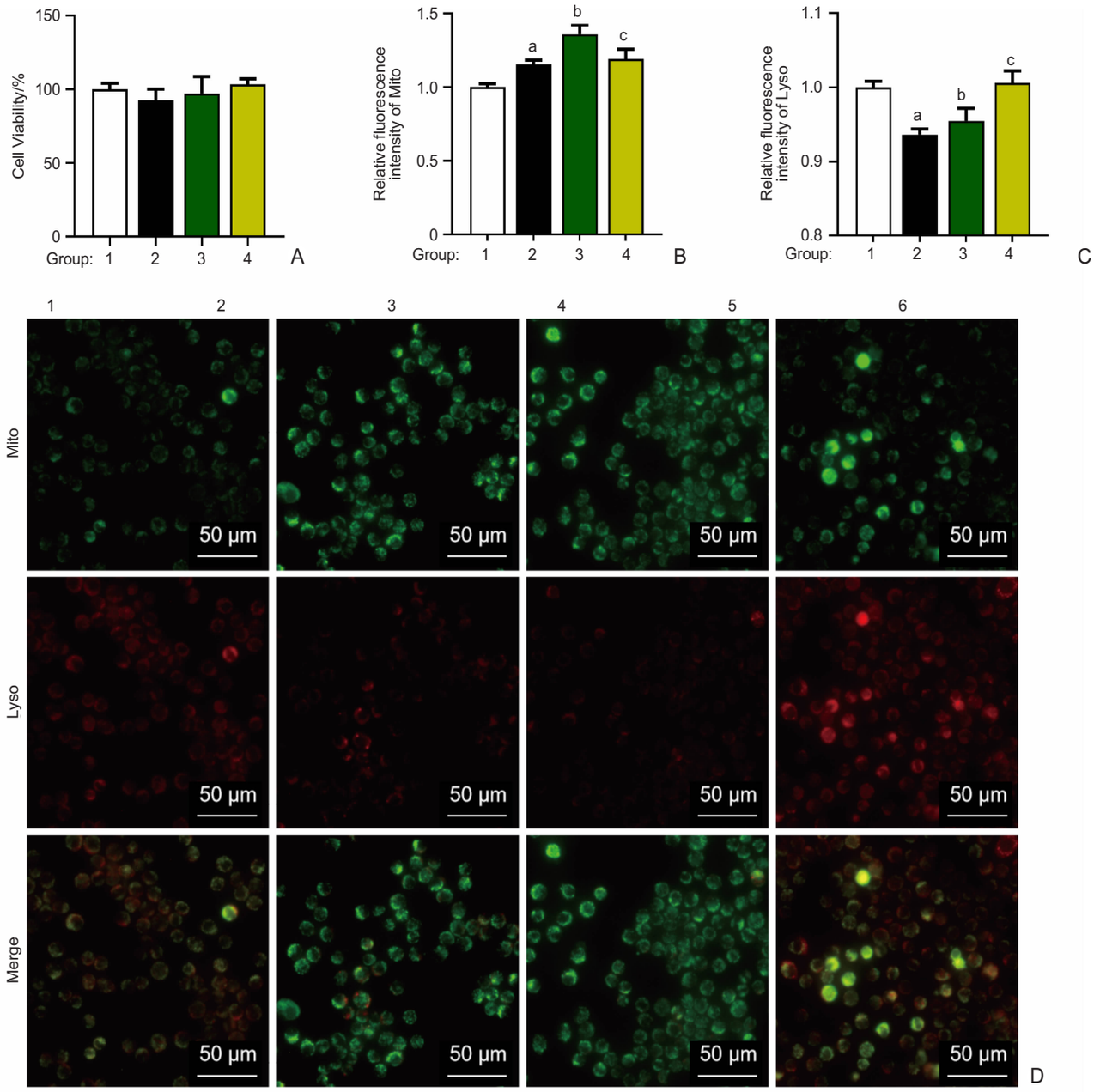
Figure 2 Effect of fructose on the mitochondrial membrane potential of M2-type macrophages



1: M0 group; 2: M2 group (20 ng/mL IL-4); 3: M2+0.04 mmol/L Fru group; 4: M2+0.20 mmol/L Fru group; 5: M2+1.00 mmol/L Fru group; 6: M2+5.00 mmol/L Fru group. a: $P<0.05$, in comparison with the M2 group; A: Western blotting results of total-PP2Ac and demethylated-PP2Ac; B: Semi-quantitative analysis of total-PP2Ac and demethylated-PP2Ac protein levels

图3 果糖对M2型巨噬细胞Total-PP2Ac及demethylated-PP2Ac蛋白的影响

Figure 3 Effects of fructose on the protein levels of total-PP2Ac and demethylated-PP2Ac in M2-type macrophages



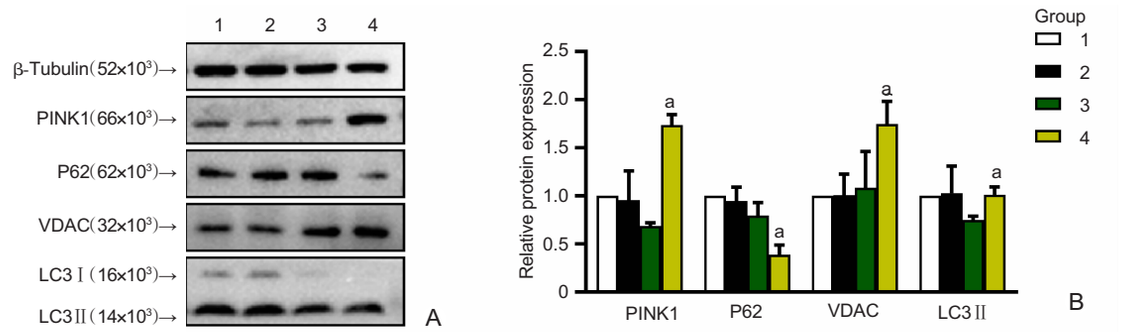
1: M0 group; 2: M2 group (20 ng/mL IL-4); 3: M2+Fru group (5.00 mmol/L Fru); 4: M2+Fru+ABL127 group (1.00 μmol/L ABL127). a: $P<0.01$, in comparison with the M0 group; b: $P<0.01$, in comparison with the M2 group; c: $P<0.01$, in comparison with the M2+Fru group; A: Cell viability after ABL127 intervention; B: Relative fluorescence intensity of mitochondria after ABL127 intervention; C: Relative fluorescence intensity of lysosomes after ABL127 intervention; D: Fluorescence changes of mitochondria (Mito) and lysosomes (Lyso) under a fluorescence microscope

图4 ABL127对果糖干预M2型巨噬细胞线粒体及溶酶体数量的影响

Figure 4 Effect of ABL127 on the numbers of mitochondria and lysosomes in M2-type macrophages after fructose exposure

2.6 ABL127缓解果糖对M2型巨噬细胞标志物mRNA表达的抑制作用

与M0组相比,M2组巨噬细胞特异性标志物*Fizz1*、*Arg-1*及*TGF-β*的mRNA水平显著上调($P<0.05$,图7),证明M2型巨噬细胞模型构建成功;与M2组相比,M2+Fru组*Fizz1*、*Arg-1*及*TGFβ*mRNA水平下降($P<0.05$);与M2+Fru组相比,M2+Fru+ABL127组中上述标志物的mRNA表达均升高($P<0.05$,图7)。结果提示果糖处理下调M2型巨噬细胞特征性标志物的基因表达,进而抑制其极化过程,而ABL127可缓解果糖对M2型巨噬细胞标志物mRNA表达的抑制作用。

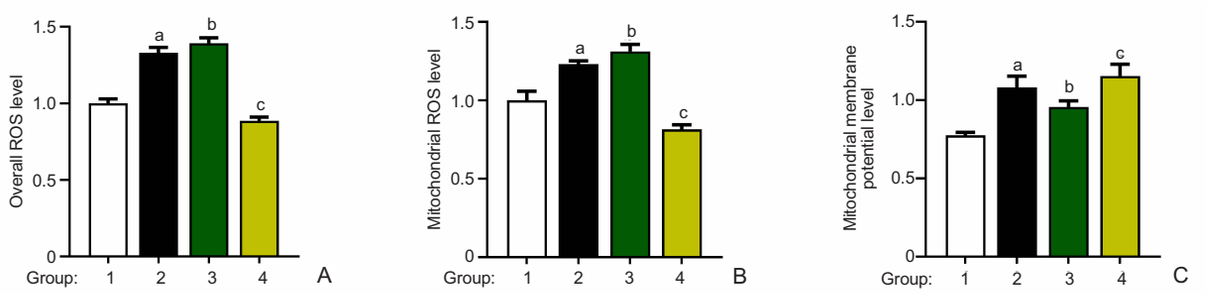


1: M0 group; 2: M2 group (20 ng/mL IL-4); 3: M2+Fru group (5.00 mmol/L Fru); 4: M2+Fru+ABL127 group (1.00 μmol/L ABL127). a: $P<0.05$, in comparison with the M2+Fru group

A: Western blotting results of PINK1, P62, VDAC and LC3Ⅱ; B: Semi-quantitative analysis of PINK1, P62, VDAC and LC3Ⅱ protein levels

图5 ABL127对果糖干预M2型巨噬细胞线粒体自噬相关蛋白表达的影响

Figure 5 Effect of ABL127 on the expression of mitophagy-related proteins in M2-type macrophages exposed to fructose

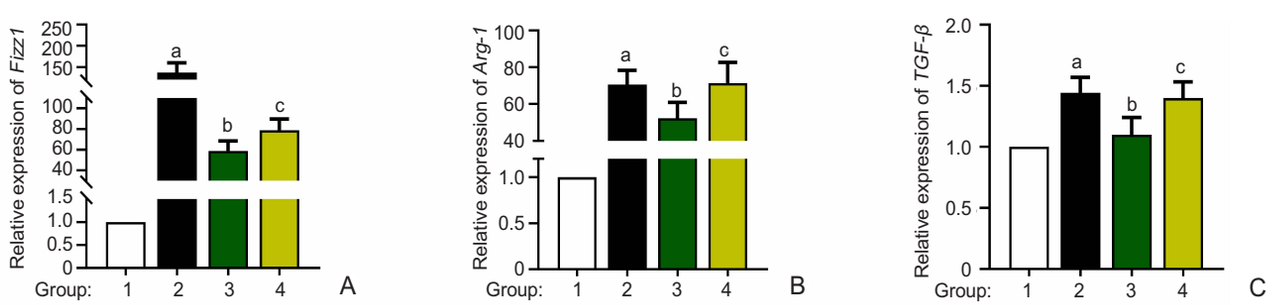


1: M0 group; 2: M2 group (20 ng/mL IL-4); 3: M2+Fru group (5.00 mmol/L Fru); 4: M2+Fru+ABL127 group (1.00 μmol/L ABL127). ROS: Reactive oxygen species. a: $P<0.01$, in comparison with the M0 group; b: $P<0.05$, in comparison with the M2 group; c: $P<0.01$, in comparison with the M2+Fru group

A: Overall ROS level after ABL127 intervention; B: Mitochondrial ROS level after ABL127 intervention; C: Mitochondrial membrane potential level after ABL127 intervention

图6 ABL127对果糖干预M2型巨噬细胞ROS及线粒体膜电位的影响

Figure 6 Effect of ABL127 on ROS level and mitochondrial membrane potential in M2-type macrophages exposed to fructose



1: M0 group; 2: M2 group (20 ng/mL IL-4); 3: M2+Fru group (5.00 mmol/L Fru); 4: M2+Fru+ABL127 group (1.00 μmol/L ABL127). a: $P<0.05$, in comparison with the M0 group; b: $P<0.05$, in comparison with the M2 group; c: $P<0.05$, in comparison with the M2+Fru group

A: Relative expression of *Fizz1* after ABL intervention; B: Relative expression of *Arg-1* after ABL intervention; C: Relative expression of *TGF-β* after ABL intervention

图7 ABL127对果糖干预M2型巨噬细胞极化标志物的影响

Figure 7 Effect of ABL127 on the polarization markers of M2-type macrophages exposed to fructose

3 讨论

3.1 膳食果糖诱导ROS蓄积干扰巨噬细胞线粒体功能与极化稳定

近年来,高果糖配比的果葡糖浆作为加工原料广泛应用于食品行业,而摄入过量果糖是诱导胰岛素抵抗、肥胖以及代谢性脂肪性肝病的重要因素[13]。既往研究多聚焦于较高浓度果糖(≥10.00 mmol/L)的体外病理机制探索。然而,该浓度显著高于膳食果糖摄入后在人机体门静脉循环中可能达到的峰值浓度(1.00~5.00 mmol/L)[14]。与既往研究使用的高浓度不同,本研究发现在0.04~5.00 mmol/L这一涵盖膳食暴露水平的浓度范围内,果糖可剂量依赖性地诱导线粒体氧化损伤,表现为总体ROS、线粒体源ROS生成增加及线粒体膜电位下降,并在果糖浓度达到5.00 mmol/L时具有显著的统计学差异。提示膳食果糖摄入增加具有潜在的线粒体氧化应激风险。

果糖代谢主要发生在富含果糖特异性激酶(ketohexokinase,KHK)的肝脏及肠道。巨噬细胞虽低表达KHK,却可通过广谱己糖激酶2(hexokinase 2,HK2)实现代谢,尽管HK2对果糖的催化效率较低,但其介导的代谢过程可通过表观遗传重编程干扰肿瘤相关巨噬细胞的极化状态,促进结直肠癌发展[15]。在本研究中,果糖可通过下调M2型巨噬细胞标志物*Fizz1*、*Arg-1*及*TGF-β*基因表达干扰其极化稳定,提示高果糖微环境可能介导巨噬细胞免疫应答及组织修复功能的紊乱。JONES等[16]研究报道,经果糖预处理的炎性单核-巨噬细胞可加速丙酮酸向线粒体三羧酸循环(tricarboxylic acid cycle,TCA)的转运以强化氧化磷酸化代谢,上调的TCA循环显著增加电子向电子传递链(electron transport chain,ETC)的供应[17]。在活化的巨噬细胞中,ETC活性的增强直接促进线粒体源ROS的产生[18]。这意味着果糖可能加剧细胞内还原当量的积累,导致ETC系统产生过量的ROS。本研究结果显示,果糖可诱导M2型巨噬细胞线粒体数量增加,同时伴随细胞ROS水平升高。这提示果糖除了增强ETC活性外,可通过促进线粒体生物合成间接加剧ROS生成。机制上,可能与果糖显著上调线粒体裂变蛋白(mitochondrial fission 1 protein,FIS1)的表达有关,其驱动线粒体过度分裂与增殖[19],最终造成整体ROS累积。

3.2 果糖通过抑制PP2A活性阻断线粒体自噬导致线粒体功能障碍

在经典理论中,线粒体膜电位下降触发PINK1蓄积于线粒体外膜,激活E3泛素-蛋白连接酶Parkin依赖性线粒体自噬[20]。然而本研究显示,5.00 mmol/L果糖处理24 h虽导致线粒体膜电位下降,但线粒体自噬启动关键分子PINK1未出现显著上调,提示果糖可在早期阻断线粒体自噬启动通路。有意思的是,最新研究指出,PINK1蛋白稳定表达于受损线粒体外膜涉及多种蛋白的协调作用,如PINK1二聚化或线粒体外膜转运蛋白(translocator of the outer mitochondrial membrane,TOM)与VDAC复合体的组装[21],果糖可能通过影响PINK1稳定相关的关键环节干扰线粒体自噬启动,并造成后续的线粒体累积。此外,果糖通过抑制AMP活化蛋白激酶导致哺乳动物雷帕霉素靶蛋白复合体1(mammalian target of rapamycin1,mTORC1)激活[22],及过量ROS氧化修饰自噬核心调控蛋白如ATG3、ATG7和SENP3[23],提示果糖可能间接抑制自噬活性。

PP2A通过动态调控多种转录因子和蛋白激酶的磷酸化状态,广泛参与细胞信号网络调控,其活性变化在抗氧化应激中起关键作用[24]。研究表明,活化PP2A可通过抑制NADPH氧化酶活性减轻氧化损伤[25],并可介导叉头框蛋白O1转录因子去磷酸化,促进其核转位以启动抗氧化应激反应和代谢调控基因的表达[26]。然而,氧化应激亦可诱导PP2Ac亚基Tyr307位点磷酸化,导致PP2A失活[27]。失活的PP2A进一步促进核因子κB依赖性氧化应激与炎症的恶性循环[28]。本研究发现,在果糖刺激下,PP2Ac的去甲基化水平呈浓度依赖性升高,提示PP2A活性逐步抑制。在先前的研究中发现巨噬细胞可通过PP2A介导的线粒体自噬途径进行代谢重编程以响应微环境改变[29]。值得注意的是,在应激或炎症时,线粒体自噬可防止受损线粒体的积累和ROS过度增加[30]。近来研究显示,PP2A可作为自噬启动的"分子开关",通过去磷酸化unc-51样激酶1(unc-51 like autophagy activating kinase 1,ULK1)关键位点解除mTORC1对自噬的抑制,直接激活自噬体形成[31]。同时活化的ULK1通过磷酸化PP2A调控亚基纹状蛋白(Striatin,STRN)形成正反馈回路,显著放大自噬信号[32]。此外,PP2A还能通过去磷酸化转录因子EB,促进其核转位,驱动溶酶体生物合成及自噬相关基因的转录激活[33]。本研究中,果糖诱导PP2Ac亚基过度去甲基化,抑制PP2A酶活性。失活的PP2A无法有效调控自噬关键节点,导致线粒体自噬启动障碍,未能及时清除多余或受损线粒体,进而引起线粒体蓄积与ROS积累加剧。ABL127通过增强PP2A活性,上调溶酶体生成及线粒体自噬相关蛋白PINK1、VDAC的表达,从而

有效清除受损线粒体,阻断了果糖诱导的ROS蓄积与线粒体氧化损伤的恶性循环。以上研究提示,PP2A作为连接营养代谢和细胞功能的一个重要信号节点,可通过调控下游的自噬通路来维持线粒体质量与细胞氧化应激平衡。

综上所述,本研究揭示了膳食果糖暴露的浓度范围内,果糖具备直接损害巨噬细胞线粒体功能、诱发氧化应激的潜力,而PP2A在其中的线粒体氧化应激损伤中起到关键作用。这些发现为理解膳食果糖相关代谢疾病的早期细胞机制提供了新的实验依据,并为开发针对代谢紊乱和氧化应激相关疾病的治疗策略提供了潜在的靶点。

利益冲突 本文所有作者声明不存在利益冲突

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