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PI3K/Akt/mTOR通路介导巨噬细胞自噬影响矽尘致肺成纤维细胞表型转化

杜悦，黄芳财，关岚，曾明

(中南大学湘雅公共卫生学院卫生毒理学系，长沙 410006)

[摘要] **目的：**磷脂酰肌醇-3-激酶-蛋白激酶B-哺乳动物雷帕霉素靶蛋白(phosphatidylinositol-3-kinase-protein kinase B-mammalian target of rapamycin，PI3K/Akt/mTOR)信号通路是自噬相关主要信号通路之一。自噬在硅沉着病纤维化形成过程中发挥关键作用。肺成纤维细胞向肌成纤维细胞表型转化是硅沉着病由炎症期进入纤维化期的标志之一。本研究旨在探讨PI3K/Akt/mTOR通路是否通过介导巨噬细胞自噬影响矽尘诱导的肺成纤维细胞向肌成纤维细胞表型转化。**方法：**采用100 ng/mL佛波酯诱导人单核细胞白血病细胞系THP-1细胞24 h获得巨噬细胞。以不同浓度(0、25、50、100、200、400 μg/mL)二氧化硅(silicon dioxide，SiO_2)粉尘悬浮液染毒巨噬细胞不同的时间(0、6、12、24、48 h)。采用细胞计数试剂盒-8(cell counting kit-8，CCK-8)法检测巨噬细胞存活率，酶联免疫吸附试验(enzyme linked immunosorbent assay，ELISA)法检测巨噬细胞上清液中转化生长因子β1(transforming growth factor-β1，TGF-β1)、肿瘤坏死因子α(tumor necrosis factor-α，TNF-α)的含量。采用Transwell技术建立巨噬细胞和HFL-1细胞共培养体系，设空白对照组、SiO_2组、LY294002组、SC79组、LY294002+SiO_2组、SC79+SiO_2组。LY294002+SiO_2组、SC79+SiO_2组分别用LY294002(PI3K抑制剂)、SC79(Akt激活剂)预处理巨噬细胞18 h、24 h，再用SiO_2(100 μg/mL)粉尘悬浮液染毒巨噬细胞12 h。采用免疫荧光法检测巨噬细胞中微管相关蛋白1轻链3(microtubule-associated protein 1 light chain 3，LC3)的表达情况；蛋白印迹法检测巨噬细胞PI3K、Akt、mTOR、Beclin-1、LC3的蛋白质表达水平及HFL-1细胞中III型胶原蛋白(collagen III，Col III)、α-平滑肌肌动蛋白(α-smooth muscle actin，α-SMA)、纤维连接蛋白(fibronectin，FN)、基质金属蛋白酶-1(matrix metalloproteinase-1，MMP-1)、金属蛋白酶组织抑制因子-1(tissue matrix metalloproteinase inhibitor-1，TIMP-1)的蛋白质表达水平。**结果：**用不同浓度的SiO_2粉尘悬浮液染毒巨噬细胞12 h，巨噬细胞的存活率随SiO_2浓度的增加逐渐降低，与0 μg/mL组比较，100、200、400 μg/mL组细胞存活率均明显降低，细胞上清液中TGF-β1和TNF-α含量均明显增加(均$P<0.05$)；用100 μg/mL SiO_2粉尘悬浮液染毒巨噬细胞，巨噬细胞的存活率随染毒时间的延长逐渐降低，与0 h组比较，6、12、24、48 h组细胞存活率明显降低(均$P<0.05$)，细胞上清液中TGF-β1和TNF-α含量均明显增加，Beclin-1和LC3II的蛋白质表达水平均明显上调(均$P<0.05$)。免疫荧光法结果显示：100 μg/mL的SiO_2粉尘处理巨噬细胞12 h后LC3的荧光呈点状聚集，且荧光强度明显高于空白对照组($P<0.05$)。与空白对照组相比，SiO_2组HFL-1细胞中Col III、FN、α-SMA、MMP-1、TIMP-1的蛋白质表达均上调(均$P<0.05$)；与SiO_2组相比，LY294002+SiO_2组中巨噬细胞PI3K、Akt、mTOR的蛋白质表达均下调(均$P<0.05$)，LC3II和Beclin-1的蛋白质表达均上调(均$P<0.05$)，细胞上清液中TGF-β1和TNF-α含量均降低(均$P<0.01$)，HFL-1细胞中Col III、FN、α-SMA、MMP-1、TIMP-1的蛋白质表达均下调(均$P<0.05$)；SC79+SiO_2组巨噬细胞PI3K、Akt、mTOR的蛋白质表达均上调(均$P<0.05$)，LC3II和Beclin-1的蛋白质表达均下调(均$P<0.05$)，细胞上清液中TGF-β1和TNF-α含量均增加(均$P<0.01$)，HFL-1细胞中Col III、FN、α-SMA、MMP-1、TIMP-1的蛋白质表达均上调(均$P<0.05$)。**结论：**矽尘染毒引起巨噬细胞的PI3K/Akt/mTOR通路抑制、自噬发生及炎症因子分泌增加，进而促进HFL-1细胞向肌成纤维细胞表型转化；调控PI3K/Akt/mTOR通路可通过影响矽尘对巨噬细胞的自噬诱导及炎症因子分泌，从而调节

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第一作者(First author)：杜悦，Email: 216912054@csu.edu.cn, ORCID: 0000-0003-2425-3347；黄芳财，Email: lizuihfc@163.com, ORCID: 0000-0002-1126-8737

通信作者(Corresponding author)：曾明，Email: zengming@csu.edu.cn, ORCID: 0000-0002-6150-8591

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HFL-1细胞向肌成纤维细胞表型转化。

[关键词] 矽尘；肺成纤维细胞；肌成纤维细胞；巨噬细胞自噬；PI3K/Akt/mTOR通路；转化生长因子β1；肿瘤坏死因子α

Role of PI3K/Akt/mTOR pathway-mediated macrophage autophagy in affecting the phenotype transformation of lung fibroblasts induced by silica dust exposure

DU Yue, HUANG Fangcai, GUAN Lan, ZENG Ming

(Department of Health Toxicology, Xiangya School of Public Health, Central South University, Changsha 410006, China)

ABSTRACT

Objective: The phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway is one of the main signaling pathways related to autophagy. Autophagy plays a key role in the formation of silicosis fibrosis. The phenotypic transformation of lung fibroblasts into myofibroblasts is a hallmark of the transition from the inflammatory phase to the fibrotic phase in silicosis. This study aims to investigate whether the PI3K/Akt/mTOR pathway affects the phenotypic transformation of silicosis-induced lung fibroblasts into myofibroblasts via mediating macrophage autophagy.

Methods: The human monocytic leukemia cell line THP-1 cells were differentiated into macrophages by treating with 100 ng/mL of phorbol ester for 24 h. Macrophages were exposed to different concentrations (0, 25, 50, 100, 200, 400 μg/mL) and different times (0, 6, 12, 24, 48 h) of SiO_2 dust suspension. The survival rate of macrophages was measured by cell counting kit-8 (CCK-8) method. Enzyme linked immunosorbent assay (ELISA) was used to measure the contents of transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α) in the cell supernatant. The co-culture system of macrophages and HFL-1 cells was established by transwell. A blank control group, a SiO_2 group, a LY294002 group, a SC79 group, a LY294002+SiO_2 group, and a SC79+SiO_2 group were set up in this experiment. Macrophages in the LY294002+SiO_2 group were pretreated with LY294002 (PI3K inhibitor) for 18 hours, and macrophages in the SC79+SiO_2 group were pretreated with SC79 (Akt activator) for 24 hours, and then exposed to SiO_2 (100 μg/mL) dust suspension for 12 hours. The expression of microtubule-associated protein 1 light chain 3 (LC3) protein in macrophages was detected by the immunofluorescence method. The protein expressions of PI3K, Akt, mTOR, Beclin-1, LC3 in macrophages, and collagen III (Col III), α-smooth muscle actin (α-SMA), fibronectin (FN), matrix metalloproteinase-1 (MMP-1), tissue metalloproteinase inhibitor-1 (TIMP-1) in HFL-1 cells were measured by Western blotting.

Results: After the macrophages were exposed to SiO_2 dust suspension of different concentrations for 12 h, the survival rates of macrophages were gradually decreased with the increase of SiO_2 concentration. Compared with the 0 μg/mL group, the survival rates of macrophages in the 100, 200, and 400 μg/mL groups were significantly decreased, and the

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concentrations of TGF-β1 and TNF-α in the cell supernatant were obviously increased (all $P<0.05$). When 100 μg/mL SiO_2 dust suspension was applied to macrophages, the survival rates of macrophages were decreased with the prolonged exposure time. Compared with the 0 h group, the survival rates of macrophages were significantly decreased (all $P<0.05$), the concentrations of TGF-β1 and TNF-α in the cell supernatant were significantly increased, and the protein expression levels of Beclin-1 and LC3II were increased markedly in the 6, 12, 24, and 48 h groups (all $P<0.05$). Immunofluorescence results demonstrated that after exposure to SiO_2 (100 μg/mL) dust for 12 h, LC3 exhibited punctate aggregation and significantly higher fluorescence intensity compared to the blank control group ($P<0.05$). Compared with the blank control group, the protein expressions of Col III, FN, α-SMA, MMP-1, and TIMP-1 in HFL-1 cells were up-regulated in the SiO_2 group (all $P<0.05$). Compared with the SiO_2 group, the protein expressions of PI3K, Akt, and mTOR were down-regulated and the protein expressions of LC3II and Beclin-1 were up-regulated in macrophages (all $P<0.05$), the contents of TNF-α and TGF-β1 in the cell supernatant were decreased (both $P<0.01$), and the protein expressions of Col III, FN, α-SMA, MMP-1, and TIMP-1 in HFL-1 cells were down-regulated (all $P<0.05$) in the LY294002+SiO_2 group. Compared with the SiO_2 group, the protein expressions of PI3K, Akt, and mTOR were up-regulated and the protein expressions of LC3II and Beclin-1 were down-regulated in macrophages (all $P<0.05$), the contents of TNF-α and TGF-β1 in the cell supernatant were increased (both $P<0.01$), and the protein expressions of Col III, FN, α-SMA, MMP-1, and TIMP-1 in HFL-1 cells were up-regulated (all $P<0.05$) in the SC79+SiO_2 group.

Conclusion: Silica dust exposure inhibits the PI3K/Akt/mTOR pathway, increases autophagy and concentration of inflammatory factors in macrophages, and promotes the phenotype transformation of HFL-1 cells into myofibroblasts. The regulation of the PI3K/Akt/mTOR pathway can affect the autophagy induction and the concentration of inflammatory factors of macrophages by silica dust exposure, and then affect the phenotype transformation of HFL-1 cells into myofibroblasts induced by silica dust exposure.

KEY WORDS silica dust; lung fibroblasts; myofibroblasts; macrophage autophagy; phosphatidylinositol-3-kinase-protein kinase B-mammalian target of rapamycin pathway; transforming growth factor-β1; tumor necrosis factor-α

硅沉着病是世界上最常见的职业病之一，因患者长期暴露于游离二氧化硅(silicon dioxide，SiO_2)粉尘中所致，其特征为肺部慢性炎症和进行性纤维化[1]。矽尘颗粒进入肺内并激活巨噬细胞，使其分泌肿瘤坏死因子α(tumor necrosis factor-α，TNF-α)、转化生长因子β1(transforming growth factor-β1，TGF-β1)等多种炎症因子，TNF-α和TGF-β1常被作为观察矽尘所致肺部炎症或纤维化发生的指标[2-3]。大量炎症因子的释放引起肺成纤维细胞增殖、活化，并向肌成纤维细胞转化，且该过程伴随细胞外基质(extracellular matrix，ECM)的过度沉积，最终导致肺纤维化[4]。肺成纤维细胞向肌成纤维细胞表型转化是硅沉着病由炎症期进入纤维化期的标志之一。抑制肺成纤维细胞表型转化可能减轻或延缓肺纤维化。

自噬包括启动、自噬体的形成、自噬体与溶酶体的融合、自噬溶酶体的降解4个过程。在硅沉着病肺纤维化发生过程中，巨噬细胞通过自噬清除尘粒[5]。自噬相关蛋白Beclin-1和微管相关蛋白1轻链3(microtubule-associated protein 1 light chain 3，LC3)常被用作评价和测定自噬的指标[6-7]。增加小鼠肺组织的自噬活性后，LC3II、Beclin-1的表达增加，SiO_2暴露所致的小鼠纤连蛋白(fibronectin，FN)、α-平滑肌肌动蛋白(α-smooth muscle actin，α-SMA)的表达水平降低，肺组织成纤维细胞的分化减弱，进而抑制肺纤维化的进展[8]。自噬在硅沉着病纤维化形成过程中发挥着关键作用。

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磷脂酰肌醇-3-激酶-蛋白激酶B-哺乳动物雷帕霉素靶蛋白(phosphatidylinositol-3-kinase-protein kinase B-mammalian target of rapamycin，PI3K/Akt/mTOR)信号通路是自噬相关主要信号通路之一。抑制PI3K/Akt/mTOR通路活化可明显增加巨噬细胞自噬[9]。抑制PI3K/Akt/mTOR信号通路转导可引起自噬相关标志物LC3II和Beclin-1的表达上调，同时肺纤维化也有所改善[10]。PI3K/Akt/mTOR信号通路可能对矽尘致肺纤维化有重要影响。

巨噬细胞自噬以及自噬相关的PI3K/Akt/mTOR信号通路与硅沉着病密切相关。然而，矽尘致肺纤维化过程涉及的通路及机制复杂，在该过程中矽尘能否激活PI3K/Akt/mTOR通路介导的巨噬细胞自噬以及自噬在肺成纤维细胞表型转化为肌成纤维细胞过程中的作用尚不清楚。因此，本研究拟探究巨噬细胞自噬通路PI3K/Akt/mTOR对矽尘致人胚肺成纤维细胞系细胞向肌成纤维细胞表型转化过程的影响，以期为深入了解硅沉着病发生的分子机制及其防治提供新的线索。

1 材料与方法

1.1 材料

游离二氧化硅粉尘(SiO_2，粒径小于5 μm)购自美国Sigma公司；LY294002(选择性PI3K抑制剂)、SC79(Akt激活剂)、ECL发光试剂盒均购自美国TargetMol公司；佛波酯(phorbol-12-myristate-13-acetate，PMA)试剂购自美国AbMole公司；CD11b-PE流式抗体购自美国eBioscience公司；细胞计数试剂盒-8(cell counting kit-8，CCK-8)购自美国Genview公司；BCA蛋白质定量试剂盒购自北京鼎国生物有限公司；TGF-β1、TNF-α的酶联免疫吸附试验(enzyme linked immunosorbent assay，ELISA)试剂盒，mTOR、LC3、Beclin-1、FN、α-SMA、基质金属蛋白酶-1(matrix metalloproteinase-1，MMP-1)、金属蛋白酶组织抑制因子-1(tissue matrix metalloproteinase inhibitor-1，TIMP-1)及III型胶原蛋白(collagen III，Col III)一抗均购自美国ABclonal公司；PI3K、Akt一抗购自美国Cell Signaling Technology公司。

1.2 方法

1.2.1 细胞的培养与诱导分化

人单核细胞白血病细胞系THP-1(THP-1)购自中国科学院干细胞库；人胚肺成纤维细胞系(HFL-1)购自中南大学高等研究中心生物细胞室。THP-1细胞培养于含10%胎牛血清(fetal bovine serum，FBS)的RPMI 1640，HFL-1细胞培养于含15% FBS的DMEM，二者均置于37 ℃、5% CO_2的培养箱中培养。诱导THP-1细胞分化为巨噬细胞：用含100 ng/mL PMA的RPMI 1640(未加FBS)诱导THP-1细胞24 h，再用含10% FBS的RPMI 1640培养48 h。

1.2.2 巨噬细胞的鉴定

CD11b是细胞黏附分子整合蛋白家族的α链，常被用作判断单核细胞分化为巨噬细胞的重要标志物。收集1.2.1中用PMA诱导分化的巨噬细胞，并以未用PMA诱导的THP-1细胞作为对照；将收集到的细胞离心后弃上清，用PBS洗涤；分别向2组细胞中加入PE-CD11b抗体，在室温下孵育1 h；用PBS洗涤后，以PBS重悬细胞，采用流式细胞仪检测CD11b的表达来鉴定巨噬细胞。

1.2.3 共培养体系的建立

采用Transwell技术建立共培养体系，即将2种细胞分别接种于上室和下室，细胞之间不直接接触，但可通过细胞分泌的细胞因子和代谢产物等进行信息交流。巨噬细胞与HFL-1细胞共培养体系：将巨噬细胞接种于6孔板细胞上室中，在6孔板细胞下室接种与巨噬细胞密度接近的HFL-1细胞；用含15% FBS的DMEM，置于37 ℃、5% CO_2的培养箱中共培养。

1.2.4 分组

为研究矽尘对巨噬细胞存活率、炎症因子分泌及自噬的影响，以不同浓度(0、25、50、100、200、400 μg/mL)和时间(0、6、12、24、48 h)将巨噬细胞暴露于SiO_2粉尘悬浮液。染毒完成后检测巨噬细胞存活率、巨噬细胞上清液中TGF-β1和TNF-α的含量、Beclin-1和LC3II的蛋白质表达水平、巨噬细胞自噬激活情况。

为研究PI3K/Akt/mTOR通路对矽尘致巨噬细胞自噬及炎症因子分泌、HFL-1细胞表型转化的影响，采用Transwell技术建立巨噬细胞和HFL-1细胞共培养体系，以100 mg/L SiO_2粉尘悬浮液染毒12 h，建立HFL-1细胞向肌成纤维细胞表型转化的模型。进行如下分组：空白对照组、SiO_2组(100 μg/mL SiO_2染毒12 h)、LY294002组(10 μmol/L LY294002处理巨噬细胞18 h)、SC79组(10 μmol/L SC79处理巨噬细胞24 h)、LY294002+SiO_2组(先用10 μmol/L LY294002处理巨噬细胞18 h，再100 μg/mL SiO_2染毒12 h)、SC79+SiO_2组(先用10 μmol/L SC79处理巨噬细胞24 h，再100 μg/mL SiO_2染毒12 h)。LY294002、SC79的预处理浓度与时间为预实验中引起自噬水平变化(以Beclin-1蛋白质表达水平判断)最明显的浓度与时间。处理完细胞后，采用蛋白质印迹法检测巨噬细胞中

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Beclin-1、LC3II、PI3K、Akt、mTOR，HFL-1细胞中Col III、FN、α-SMA、MMP-1、TIMP-1的表达情况；ELISA检测细胞上清液中TGF-β1和TNF-α含量。

1.2.5 CCK-8法

采用CCK-8法检测巨噬细胞存活率。将巨噬细胞(5×10⁴个/孔)接种于96孔板中，染毒完成后每孔加入10 μL CCK-8并混匀，继续培养1.5 h；采用酶标仪测量450 nm波长处每孔的吸光度值，并以染毒浓度为0 μg/mL作为参照，计算细胞存活率。

1.2.6 ELISA法

采用ELISA检测巨噬细胞上清液中TGF-β1、TNF-α的含量。将巨噬细胞接种于6孔板中，染毒完成后取细胞上清液并过滤；严格按照ELISA试剂盒说明书进行操作，采用酶标仪测量450 nm波长处每孔的吸光度值，TGF-β1、TNF-α的校正波长分别为570、630 nm。绘制标准曲线图，计算TGF-β1、TNF-α含量。

1.2.7 蛋白质印迹法

采用蛋白质印迹法检测蛋白质表达水平。分别收集各组细胞，加入细胞裂解液提取蛋白质，用BCA蛋白质定量试剂盒测定蛋白质浓度。配制8%~15%的SDS-PAGE分离胶，5%的SDS-PAGE浓缩胶，上样并进行电泳和转膜反应。用5%脱脂奶粉封闭1 h，1×TBST洗涤后，加入一抗在4 ℃环境中孵育过夜。针对巨噬细胞所使用的一抗有PI3K、Akt、mTOR、LC3、Beclin-1；针对HFL-1细胞所使用的一抗有Col III、FN、α-SMA、MMP-1、TIMP-1。用1×TBST洗涤后，加入二抗在室温下孵育1 h，用ECL化学发光液进行显色，并以ImageJ软件分析蛋白质的相对表达水平。

1.2.8 免疫荧光法

采用免疫荧光法检测巨噬细胞自噬激活情况。将处理后的巨噬细胞用4%多聚甲醛在室温下固定20 min，用PBS清洗3次，每次5 min；加入0.1% TritonX-100在室温下通透20 min，PBS清洗3次，每次5 min；加入5%牛血清白蛋白溶液在室温下封闭1 h；加入LC3一抗，在4 ℃下孵育过夜，用PBS清洗3次，每次5 min；加入荧光二抗，避光于室温下孵育1 h，PBS清洗3次，每次5 min；用4',6-二脒基-2-苯基吲哚二盐酸盐(4',6-diamidino-2-phenylindole，DAPI)复染核5 min，PBS清洗3次，每次5 min，于荧光显微镜下观察并采集图像。

1.3 统计学处理

采用统计学软件SPSS 21.0进行数据分析，计量资料以均数±标准差($\bar{x}\pm s$)表示。不同数据间的比较采用单因素方差分析，多组间的两两比较用LSD-t检验，P<0.05为差异有统计学意义。

2 结 果

2.1 PMA诱导THP-1细胞分化为巨噬细胞

在倒置显微镜下可见经PMA诱导后的THP-1细胞，形态上由球形的悬浮细胞分化为形状不规则的贴壁细胞并长出伪足(图1A)，具有巨噬细胞的形态特征。相比未用PMA诱导的THP-1细胞，PMA诱导后的THP-1细胞上CD11b阳性表达率明显升高(P<0.05，图1B)。以上结果表明，THP-1细胞已分化为巨噬细胞，可用于后续研究。

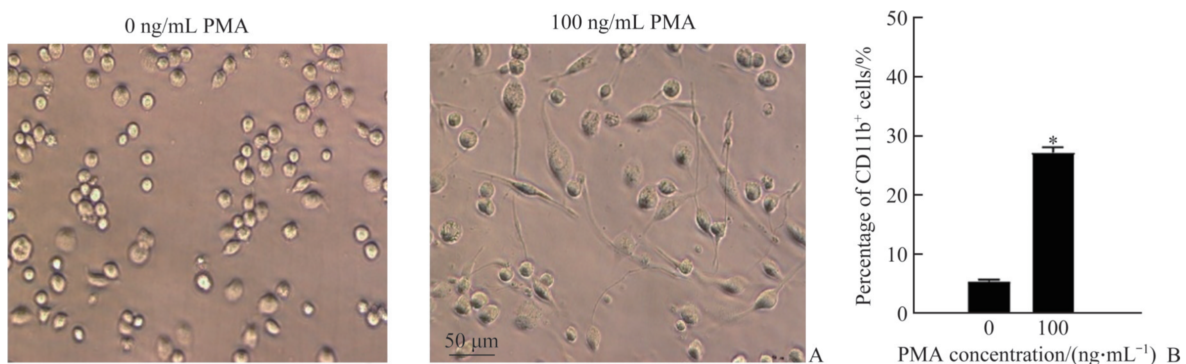

图1 PMA诱导THP-1细胞分化为巨噬细胞

Figure 1 PMA induces THP-1 cell differentiation into macrophages

A: Cell morphology under an inverted microscope. After treatment with 100 ng/mL PMA, THP-1 cells differentiated from spherical suspension cells to irregularly shaped adherent cells and grew pseudopodia. B: Percentage of $CD11b^+$ cells detected by flow cytometry. The percentage of $CD11b^+$ in THP-1 cells treated with 100 ng/mL PMA is significantly higher than that in the 0 ng/mL PMA group (n=3, $\bar{x}\pm s$). *P<0.05 vs the 0 ng/mL group. PMA: Phorbol-12-myristate-13-acetate.

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2.2 矽尘降低巨噬细胞存活率，促进炎症因子分泌

CCK-8法结果显示：用不同浓度的SiO_2粉尘悬浮液染毒巨噬细胞12 h，随着染毒浓度的增加，巨噬细胞的存活率逐渐降低。与0 μg/mL组比较，100、200、400 μg/mL组细胞存活率均明显降低(均P<0.05)，且400 μg/mL组细胞存活率低于75%(图2A)。用100 μg/mL SiO_2粉尘悬浮液染毒巨噬细胞不同时间。随着染毒时间的延长，巨噬细胞的存活率逐渐降低。与0 h组比较，6、12、24、48 h组细胞存活率明显降低(均P<0.05)，且48 h组细胞存活率低于75%(图2B)。ELISA结果显示：用不同浓度的SiO_2粉尘悬浮液染毒巨噬细胞12 h，随着染毒浓度的增加，细胞上清液中TGF-β1和TNF-α含量先增加后减少，与0 μg/mL组比较，差异均有统计学意义(均P<0.05，图2C)。用100 μg/mL SiO_2粉尘悬浮液染毒巨噬细胞不同时间，随着染毒时间的延长，TGF-β1含量先增加后减少，在12 h时含量最多(均P<0.05，图2D)，TNF-α含量随染毒时间延长而增加，与0 h组比较，差异均有统计学意义(均P<0.05，图2E)。

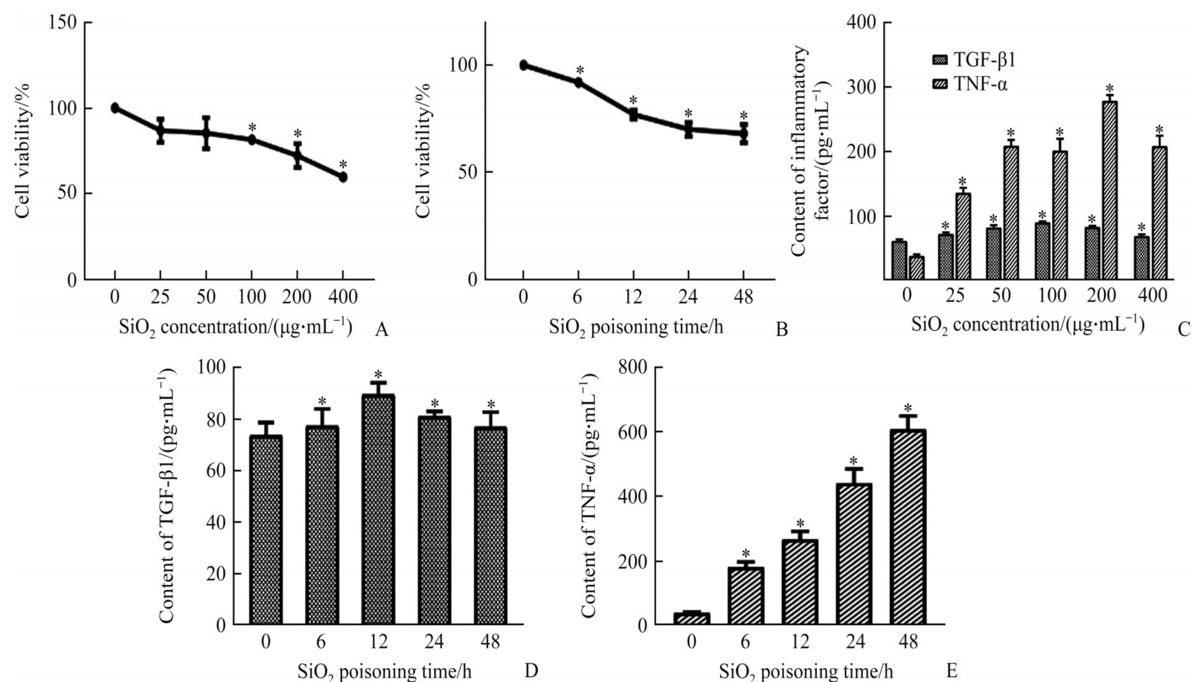

图2　矽尘对巨噬细胞存活率及炎症因子分泌的影响(n=3，$\bar{x}\pm s$)

Figure 2　Effects of SiO_2 on the cell viability and the secretion of inflammatory factors of macrophages (n=3, $\bar{x}\pm s$)

A: Effects of various concentrations of SiO_2 on the cell viability of macrophages; B: Effects of various exposure times of 100 μg/mL SiO_2 on the cell viability of macrophages; C: Effects of various concentrations of SiO_2 on the secretion of TGF-β1 and TNF-α by macrophages; D and E: Effects of various exposure time of 100 μg/mL SiO_2 on the secretion of TGF-β1 (D) and TNF-α (E) by macrophages. *P<0.05 vs the 0 μg/mL group or the 0 h group. SiO_2: Silicon dioxide; TGF-β1: Transforming growth factor-β1; TNF-α: Tumor necrosis factor-α.

2.3 矽尘激活巨噬细胞自噬

在0~24 h内，Beclin-1的蛋白质表达水平随矽尘染毒时间延长而逐渐上调，在48 h时略有下调，LC3II的蛋白质表达水平在48 h时最高；6、12、24、48 h组Beclin-1和LC3II的蛋白质表达水平与0 h组比较，差异均有统计学意义(均P<0.05，图3A)。免疫荧光法结果(图3B)显示：100 μg/mL的SiO_2粉尘处理巨噬细胞12 h后(SiO_2组)LC3的荧光呈点状聚集，而空白对照组荧光分散在巨噬细胞中；SiO_2组LC3的荧光强度明显高于空白对照组(P<0.05，图3C)。

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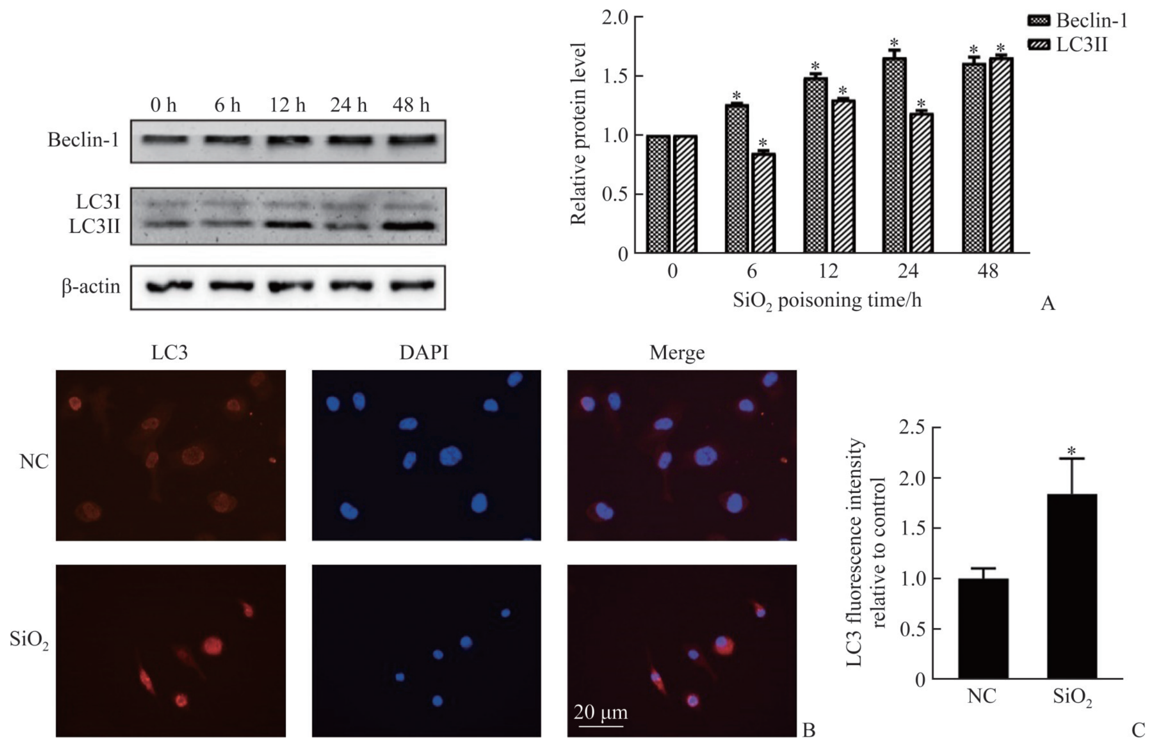

图3 矽尘对巨噬细胞自噬的影响

Figure 3 Effect of SiO_2 on autophagy in macrophages

A: After treatment with 100 μg/mL SiO_2 for 0, 6, 12, 24, and 48 hours, relative protein levels of Beclin-1 and LC3II were detected by Western blotting. The data are represented as mean±standard deviation (n=3). *P<0.05 vs the 0 h group. B and C: After treatment with 100 μg/mL SiO_2 for 12 hours, immunofluorescence shows that the fluorescence of LC3 exhibited dot like aggregation (B) and the fluorescence intensity of LC3 was significantly increased in macrophages (C). The data are represented as mean±standard deviation (n=3). *P<0.05 vs the blank control (NC) group. SiO_2: Silicon dioxide; LC3: Microtubule-associated protein 1 light chain 3; TGF-β1: Transforming growth factor-β1; TNF-α: Tumor necrosis factor-α.

2.4 PI3K/Akt/mTOR信号通路对矽尘致巨噬细胞自噬、炎症因子分泌的影响

与SiO_2组相比，LY294002+SiO_2组中巨噬细胞PI3K、Akt、mTOR的蛋白质表达均下调(均P<0.05)，LC3II和Beclin-1的蛋白质表达均上调(均P<0.05)，细胞上清液中TGF-β1和TNF-α含量均降低(均P<0.01)；SC79+SiO_2组巨噬细胞PI3K、Akt、mTOR的蛋白质表达均上调(均P<0.05)，LC3II和Beclin-1的蛋白质表达均下调(均P<0.05)，细胞上清液中TGF-β1和TNF-α含量均升高(均P<0.01，图4)。

2.5 PI3K/Akt/mTOR信号通路对矽尘致HFL-1细胞表型转化的影响

与空白对照组相比，SiO_2组HFL-1细胞中Col III、FN、α-SMA、MMP-1、TIMP-1的蛋白质表达均上调(均P<0.05)。与SiO_2组相比，SC79+SiO_2组HFL-1细胞中Col III、FN、α-SMA、MMP-1、TIMP-1的蛋白质表达均上调，而LY294002+SiO_2组HFL-1细胞中上述蛋白质表达均下调(均P<0.05，图5)。

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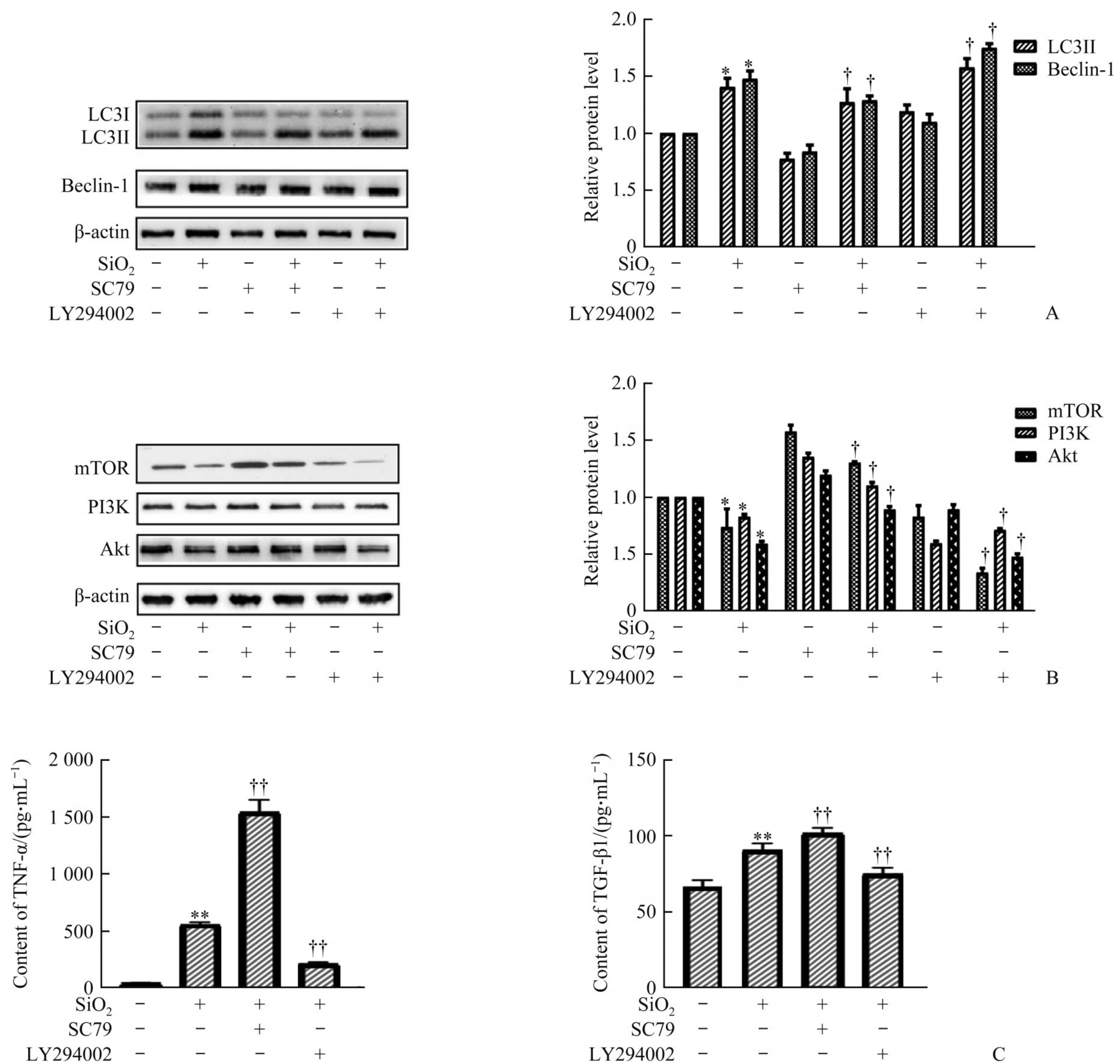

图4　LY294002通过抑制PI3K/Akt/mTOR通路促进巨噬细胞自噬，减少TGF-β1和TNF-α的分泌，而SC79通过激活PI3K/Akt/mTOR通路抑制巨噬细胞自噬，增加TGF-β1和TNF-α的分泌

Figure 4　LY294002 promotes autophagy in macrophages and reduces the secretion of TGF-β1 and TNF-α via inhibiting PI3K/Akt/mTOR pathway, and SC79 inhibits autophagy in macrophages and increases the secretion of TGF-β1 and TNF-α via activating PI3K/Akt/mTOR pathway

A: Beclin-1 and LC3 protein expression level detected by Western blotting; B: PI3K, Akt, and mTOR protein expression level detected by Western blotting; C: Content of TGF-β1 and TNF-α detected by ELISA. The data are represented as mean±standard deviation (n=3). *P<0.05, **P<0.01 vs the blank control group; †P<0.05, ††P<0.01 vs the SiO_2 group. SiO_2: Silicon dioxide; PI3K: Phosphoinositide 3-kinase; Akt: Protein kinase B; mTOR: Mammalian target of rapamycin; LC3: Microtubule-associated protein 1 light chain 3; TGF-β1: Transforming growth factor-β1; TNF-α: Tumor necrosis factor-α.

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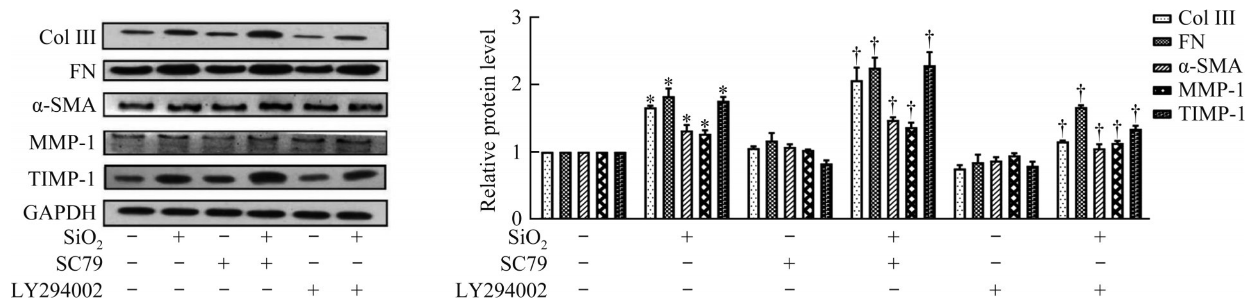

图5 LY294002抑制矽尘致HFL-1细胞表型转化为肌成纤维细胞，SC79促进矽尘致HFL-1细胞表型转化为肌成纤维细胞

Figure 5 LY294002 inhibits silica dust induced phenotype transformation of HFL-1 cells into myofibroblasts, and SC79 promotes silica dust induced phenotype transformation of HFL-1 cells into myofibroblasts

Protein expression levels of Col III, FN, α-SMA, MMP-1, and TIMP-1 detected by Western blotting. The data are represented as mean±standard deviation (n=3). *P<0.05 vs the blank control group; †P<0.05 vs the SiO_2 group. SiO_2: Silicon dioxide; Col III: Collagen III; FN: Fibronectin; α-SMA: α-Smooth muscle actin; MMP-1: Matrix metalloproteinase-1; TIMP-1: Tissue matrix metalloproteinase inhibitor-1.

3 讨 论

硅沉着病是纤维化性呼吸道疾病，且矽尘致纤维化的机制复杂。尘肺患者肺灌洗回收液中巨噬细胞数量随病情加重而明显增加，且大多数为吞尘巨噬细胞，提示巨噬细胞是矽尘颗粒作用的主要靶细胞[11]。矽尘刺激巨噬细胞分泌TGF-β1、TNF-α等炎症因子，促进肺成纤维细胞的迁移、增殖并向肌成纤维细胞分化，是肺纤维化发生和发展过程中的重要环节[12]。本研究采用Transwell技术模拟人体肺内环境，探讨矽尘刺激对巨噬细胞分泌炎症因子、自噬相关蛋白及相关信号转导通路的影响，及其对肺成纤维细胞表型转化为肌成纤维细胞的作用。

研究[13]认为炎症因子引起的肺成纤维细胞向肌成纤维细胞表型转化过程开始的标志是α-SMA的表达，且表达量与肺纤维化程度成正比。ECM沉积是肺纤维化过程的关键环节之一，硅沉着病中ECM主要在肺成纤维细胞活化、增殖、转化过程中产生[14]。FN是ECM的重要组成部分，在肺成纤维细胞转化为肌成纤维细胞过程中FN的表达和分泌增加[15]。在肺纤维化发生的早期，胶原蛋白的表达增加，以Col III表达升高为主，因此Col III是纤维化发生早期的敏感指标[16]。基质金属蛋白酶/组织基质金属蛋白酶抑制剂(matrix metalloproteinases/tissue matrix metalloproteinase inhibitor，MMPs/TIMPs)是肺纤维化进程中影响ECM降解的最重要的因子。肺纤维化早期MMPs表达增加，随着TIMPs表达的逐渐增多，胶原不断沉积，肺纤维化加重[17]。本研究结果显示：矽尘引起巨噬细胞存活率降低以及巨噬细胞分泌的TGF-β1和TNF-α明显增加，并具有一定时间和剂量依赖关系，且矽尘导致HFL-1细胞中α-SMA、FN、Col III、MMP-1、TIMP-1的表达明显增加。说明矽尘能够促使巨噬细胞分泌炎症因子并导致HFL-1细胞向肌成纤维细胞表型转化。

自噬是一种保护细胞稳态的核心分子途径[18]。为维持细胞稳态以应对矽尘的刺激，巨噬细胞发生自噬。体外试验[19]发现SiO_2暴露可导致巨噬细胞自噬活性增强。本研究结果显示矽尘引起巨噬细胞中LC3II、Beclin-1的表达增加，巨噬细胞中LC3荧光聚团呈点状分布，荧光强度明显增高，说明矽尘染毒能够引起巨噬细胞自噬。PI3K/Akt/mTOR是调节自噬的重要细胞内信号转导通路。LY294002可通过有效抑制PI3K活化来激活自噬[20]。SC79作为Akt的激动剂，能够激活小鼠巨噬细胞PI3K/Akt/mTOR通路，降低自噬相关蛋白LC3II的表达[21]。本研究为确定矽尘所激活的巨噬细胞自噬是否为PI3K/Akt/mTOR通路介导，分别使用LY294002和SC79对巨噬细胞预处理后进行矽尘染毒。结果显示：矽尘引起巨噬细胞PI3K、Akt、mTOR蛋白质表达水平下调，自噬水平升高，HFL-1细胞表型转化相关蛋白质表达上调。表明矽尘能够通过抑制PI3K/Akt/mTOR通路激活自噬，还可促进HFL-1细胞转化，与Li等[22]发现PI3K/Akt/mTOR信号通路参与SiO_2引起巨噬细胞自噬改变的结果一致。矽尘暴露引起巨噬细胞自噬的激活可能是减轻矽尘细胞毒性和促炎反应的一种应急保护机制。SC79预处理抑制巨噬细胞自噬，增加炎症因子分泌进而引起HFL-1细胞表型转化相关蛋白质表达明显上调；而LY294002预处理对矽尘染毒巨噬细胞的作用相反。这些结果表明矽尘所激活的巨噬细胞自噬是由PI3K/Akt/mTOR通路介导的。

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SC79通过激活PI3K/Akt/mTOR通路抑制巨噬细胞自噬，增加炎症因子分泌，促进HFL-1细胞向肌成纤维细胞表型转化。Jessop等[23]在体内和体外试验中发现，在矽尘暴露条件下，巨噬细胞自噬活性受损或降低的处理组与巨噬细胞自噬活性正常的处理组相比会出现更严重的炎症反应及更多的胶原沉积。这与本研究结果一致。然而，也有研究[24]认为激活巨噬细胞自噬会促进硅沉着病纤维化发展，这可能由于巨噬细胞自噬表达水平与疾病进展阶段有关。在硅沉着病早期，LC3II和Beclin-1表达增加，自噬活跃；随着疾病的进展，LC3II和Beclin-1的表达水平逐渐降低，自噬减弱[25]。

本研究显示矽尘通过抑制巨噬细胞PI3K/Akt/mTOR通路，使巨噬细胞自噬增加，促进HFL-1细胞表型转化；调控PI3K/Akt/mTOR通路能影响矽尘对巨噬细胞的自噬诱导及炎症因子分泌，进而在矽尘致HFL-1细胞向肌成纤维细胞表型转化过程中发挥作用。本研究尚有不足之处，仅检测矽尘染毒后某一时间点巨噬细胞自噬表达情况，未能动态监测矽尘染毒后不同时间点巨噬细胞自噬水平的变化。因此巨噬细胞自噬在硅沉着病中的作用和机制仍需今后深入研究。

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利益冲突声明：作者声称无任何利益冲突。

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