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研究论文

红景天苷对脂多糖诱导大鼠肺泡巨噬细胞和II型肺泡上皮细胞共培养炎性介质分泌的影响

蔡艳春[1]，黄 倩[2]，危晓莉[1]，梅汝焕[1]，撒丽娜[1]，胡晓兰[1,*]

浙江大学[1]医学院生理学系，杭州 310058；[2]第一附属医院，杭州 310006

摘 要：本研究旨在探讨红景天苷(salidroside, Sal)对脂多糖(lipopolysaccharide, LPS)诱导大鼠肺泡巨噬细胞NR 8383和II型肺泡上皮细胞RLE-6TN共培养炎性活化的影响。CCK-8比色法检测细胞增殖百分率，Western blot检测磷酸化AKT (p-AKT)和总AKT蛋白表达，酶联免疫吸附法测定细胞培养上清中肿瘤坏死因子α (tumor necrosis factor α, TNF-α)、巨噬细胞炎性蛋白2 (macrophage inflammatory protein-2, MIP-2)和白介素10 (interleukin-10, IL-10)的含量。结果显示：与对照组相比，32和128 μg/mL Sal预处理RLE-6TN细胞或共培养RLE-6TN和NR 8383细胞1 h后继续培养24 h，细胞增殖百分率显著增加($P < 0.05$)；与对照组相比，32和128 μg/mL Sal预处理RLE-6TN细胞，p-AKT/AKT蛋白比值显著增加($P < 0.05$)。32 μg/mL Sal预处理不仅抑制LPS诱导NR 8383细胞分泌TNF-α和MIP-2 ($P < 0.05$)，而且加强RLE-6TN和NR 8383细胞共培养对LPS诱导NR 8383细胞分泌TNF-α和MIP-2的抑制作用($P < 0.05$)。此外，32 μg/mL Sal预处理能促进LPS诱导NR 8383细胞分泌IL-10 ($P < 0.05$)，并能加强RLE-6TN和NR 8383细胞共培养对LPS诱导NR 8383细胞分泌IL-10的促进作用($P < 0.05$)。以上结果提示，Sal不仅能直接抑制LPS诱导的NR 8383炎性活化，还可能通过PI3K/AKT信号通路促进RLE-6TN增殖，参与II型肺泡上皮细胞对LPS诱导肺泡巨噬细胞炎性活化的调节作用。

关键词：红景天苷；脂多糖；肺泡巨噬细胞；II型肺泡上皮细胞
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Effects of salidroside on the secretion of inflammatory mediators induced by lipopolysaccharide in the co-culture of rat alveolar macrophages and type II alveolar epithelial cells

CAI Yan-Chun[1], HUANG Qian[2], WEI Xiao-Li[1], MEI Ru-Huan[1], SA Li-Na[1], HU Xiao-Lan[1,*]

[1]*Department of Physiology, School of Medicine, Zhejiang University, Hangzhou 310058, China;* [2]*The First Affiliated Hospital, Zhejiang University, Hangzhou 310006, China*

Abstract: The aim of the present study was to investigate the effect of salidroside (Sal) on inflammatory activation induced by lipopolysaccharide (LPS) in the co-culture of rat alveolar macrophages (AM) NR 8383 and type II alveolar epithelial cells (AEC II) RLE-6TN. CCK-8 colorimetric method was used to detect cell proliferation percentage. The enzyme-linked immunosorbent assay (ELISA) was used to determine the content of tumor necrosis factor alpha (TNF-α), macrophage inflammatory protein-2 (MIP-2) and interleukin-10 (IL-10) in the supernatant. Western blot was used to examine the expression levels of phosphorylated AKT (p-AKT) and total AKT protein. The results showed that pretreatment of RLE-6TN cells or co-culture of RLE-6TN and NR 8383 cells with 32 and 128 μg/mL Sal for 1 h, followed by continuous culture for 24 h, significantly increased the cell proliferation ($P < 0.05$). Compared with control group, 32 and 128 μg/mL Sal pretreatment significantly increased the ratio of p-AKT/AKT in RLE-6TN cells ($P < 0.05$). Pretreat-

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*Corresponding author. Tel: +86-571-88208517; E-mail: huxiaolan@zju.edu.cn

ment of 32 μg/mL Sal not only inhibited the secretion of TNF-α and MIP-2 by NR 8383 cells induced by LPS ($P < 0.05$), but also enhanced the inhibitory effect of RLE-6TN and NR 8383 cells co-culture on the secretion of TNF-α and MIP-2 by NR 8383 cells induced by LPS ($P < 0.05$). In addition, 32 μg/mL Sal pretreatment promoted LPS-induced IL-10 secretion by NR 8383 cells ($P < 0.05$), and enhanced the promoting effect of co-culture of RLE-6TN and NR 8383 cells on the IL-10 secretion by LPS-induced NR 8383 cells ($P < 0.05$). In conclusion, Sal may directly inhibit LPS-induced inflammatory activation of AM (NR 8383), promote the proliferation of AEC II (RLE-6TN) through PI3K/AKT signaling pathway, and enhance the regulatory effect of AEC II on LPS-induced inflammatory activation of AM.

Key words: salidroside; lipopolysaccharide; alveolar macrophages; type II alveolar epithelial cells

肺泡巨噬细胞 (alveolar macrophage, AM) 是肺内炎症反应的核心细胞，脂多糖 (lipopolysaccharide, LPS) 通过诱导 AM 过度激活产生各种炎性因子和趋化因子，触发肺内炎症级联反应，直接损伤肺实质细胞如肺泡上皮细胞 (alveolar epithelial cell, AEC) [1, 2]。AEC 包括 I 型肺泡上皮细胞 (AEC I) 和 II 型肺泡上皮细胞 (AEC II)，AEC I 覆盖了大部分肺泡表面，负责气体交换，但无增殖能力，一旦受损难以自我修复；AEC II 具有分裂增殖能力，不仅能自我更新，还可分化为 AEC I，修复受损肺泡上皮，维持肺泡上皮的完整性 [3, 4]。有研究表明，促进 AEC II 增殖和迁移的因素能减轻各种原因引起的肺损伤 [5–7]。

红景天苷 (salidroside, Sal) 为多年生草本植物红景天 (*Rhodiola*) 的主要活性成分，具有多种药理作用，对不同原因引起的器官损伤具有保护作用 [8–10]。本研究组以及其他研究小组在动物水平证实 Sal 对脂多糖 (lipopolysaccharide, LPS) 诱导的急性肺损伤 (acute lung injury, ALI) 具有保护作用 [11, 12]，但其抗 ALI 的具体细胞机制尚不清楚，是否通过调节 AEC II 发挥保护作用值得探讨。因此，本研究以大鼠 AM 细胞系 NR 8383 和 AEC II 细胞系 RLE-6TN 为研究对象，建立细胞共培养体系，探讨 Sal 对 LPS 诱导 AM 和 AEC II 共培养炎性活化的影响。

1 材料与方法

1.1 主要材料和试剂 NR 8383 和 RLE-6TN 购自美国模式培养物集存库 (American Type Culture Collection, ATCC)。LPS (*Escherichia coli* 055:B5) 和 Sal 购自 Sigma 公司。胎牛血清、DMEM 培养液、0.25% 胰酶 -EDTA 购自 Gibco 公司。CCK-8 测定试剂盒购自南京建成生物公司。细胞蛋白抽提试剂盒和 BCA 蛋白定量试剂盒购自 Pierce 公司。大鼠肿瘤坏死因子 α (tumor necrosis factor α, TNF-α)、巨噬细胞炎性蛋白 -2 (macrophage inflammatory protein-2, MIP-2) 和白介素 10 (interleukin-10, IL-10) 测定试剂盒购自 R&D 公司。兔抗鼠多克隆 AKT、p-AKT 和 β-actin 一抗以及辣根过氧化物酶标记的羊抗兔多克隆 IgG 二抗均购自 Abcam 公司。

1.2 细胞培养和共培养体系 大鼠 NR 8383 和 RLE-6TN 细胞系用含 15% 胎牛血清、2 mmol/L 谷氨酰胺、5 mmol/L 丙酮酸钠和 25 mmol/L HEPES 的 DMEM 培养液培养，其中含有 100 UI/mL 青霉素与 100 μg/mL 链霉素，放置于培养箱中，在 37 ℃、5% CO_2 饱和湿度条件下生长。0.25% 胰酶 -EDTA 消化传代，取对数生长期的细胞用于实验。细胞共培养体系的建立参考 Rubovitch 等方法 [13]：RLE-6TN 细胞传代培养，长至单层，加入 NR 8383 细胞共培养 2 h (NR 8383:RLE-6TN 为 1:5 的细胞比例)。

1.3 CCK-8 比色法测定 细胞接种于 96 孔培养板，每孔培养基总体积为 200 μL。不同剂量 Sal (0、2、8、32、128 μg/mL) 预处理 NR 8383 或 / 和 RLE-6TN 细胞 1 h，继续孵育至指定时间 (0、6、12、24 h)，加入 CCK-8 试剂 0.01 mL，置入培养箱孵育 4 h；取出后在酶标仪于 450 nm 波长处测定每孔的光密度 OD 值。细胞增殖百分率 = 处理组 OD 值 / 对照组 OD 值 × 100%。

1.4 Western blot 不同剂量 Sal (0、2、8、32、128 μg/mL) 预处理 RLE-6TN 细胞 1 h 继续培养 12 h 后收集细胞，按照蛋白提取试剂盒操作步骤提取细胞蛋白，BCA 法测定蛋白含量；50 μg 蛋白样品加上样缓冲液煮沸 5 min、取样品上样，在 8% SDS- 聚丙烯酰胺凝胶中分离后，转移到硝酸纤维素膜上。5% 脱脂奶封闭 1 h，用一抗 AKT (1:500)、p-AKT (1:500) 或 β-actin 抗体 (1:1 000) 4 °C 封闭过夜，PBS-T 洗 3 次，加入二抗 (1:3 000) 室温孵育 1 h，PBS-T 洗 3 次。用 G:BOX 化学发光成像仪，曝光目标蛋白条带，图像定量分析。

1.5 酶联免疫吸附测定 (ELISA) Sal (32 μg/mL)

预处理 NR 8383 或 / 和 RLE- 6TN 细胞 1 h 后，LPS (1 μg/mL) 刺激细胞 24 h，收集细胞培养上清。采用 ELISA 方法检测收集的细胞上清中 TNF-α、MIP-2 和 IL-10 含量，检测步骤均按照试剂盒说明书操作。

1.6 统计学分析 采用 SPSS 17.0 统计软件进行分析，计量资料以 mean ± SD 表示，多组间比较采用单因素方差分析 (one-way ANOVA)，组间两两比较采用最小显著性差异法 (LSD 法)，以 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 Sal和LPS处理对大鼠RLE-6TN或/和NR 8383细胞增殖活力的影响

不同剂量 Sal (0、2、8、32、128 μg/mL) 预处理 NR 8383 细胞 1 h，不同时间点 (0、6、12、24 h) 细胞增殖百分率无显著性差异 ($P > 0.05$，结果未展示)。这一剂量范围的 Sal 预处理 RLE-6TN 细胞，与对照组相比，32 和 128 μg/mL Sal 预处理组的细胞增殖百分率在 12 h 和 24 h 时显著增高 ($P < 0.05$，表 1)。这一剂量范围的 Sal 预处理共培养 RLE-6TN 和 NR 8383 细胞 1 h，与对照组相比，32 和 128 μg/mL Sal 预处理组的细胞增殖百分率在 24 h 时显著增高 ($P < 0.05$，表 2)。1 μg/mL LPS 处理 RLE-6TN

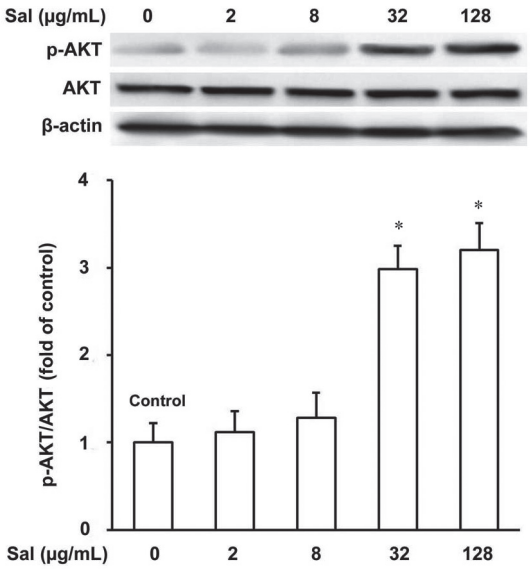

图 1. 红景天苷对RLE-6TN细胞p-AKT与总AKT比值的影响

Fig. 1. Effects of salidroside (Sal) on the ratio of p-AKT to total AKT in RLE-6TN cells. After 1 h of 0, 2, 8, 32 or 128 μg/mL Sal pretreatment, cells were cultured for another 12 h, and Western blot was used to detect the expression levels of p-AKT (56 kDa), total AKT (56 kDa) and β-actin (43 kDa) in RLE-6TN cells. The uppers were the representative results of Western blot, and the lowers were the ratio of p-AKT to total AKT, normalized to β-actin. The data are expressed as the mean ± standard deviation of six independent experiments. $^{*}P < 0.05$ *vs* control group.

表1. 红景天苷对RLE-6TN细胞增殖百分率的影响

Table 1. Effects of salidroside (Sal) on the cell proliferation percentage of RLE-6TN cells

Sal (μg/mL)	Time (h)			
	0	6	12	24
0	(100.82 ± 5.12)%	(98.11 ± 6.20)%	(100.22 ± 4.21)%	(99.35 ± 5.37)%
2	(99.24 ± 4.33)%	(99.21 ± 5.32)%	(100.63 ± 5.89)%	(102.47 ± 4.25)%
8	(100.42 ± 5.69)%	(101.72 ± 6.38)%	(103.78 ± 6.27)%	(104.33 ± 5.78)%
32	(99.45 ± 6.78)%	(103.84 ± 7.25)%	(108.58 ± 5.23)%$^{*\#}$	(110.49 ± 5.64)%$^{*\#}$
128	(101.21 ± 6.37)%	(104.47 ± 7.29)%	(109.47 ± 4.68)%$^{*\#}$	(111.58 ± 6.27)%$^{*\#}$

Mean ± SD, $n = 8$. $^{*}P < 0.05$ *vs* 0 h group at the same dose level; $^{\#}P < 0.05$ *vs* 0 dose group at the same time level.

表2. 红景天苷对共培养RLE-6TN和NR 8383细胞增殖百分率的影响

Table 2. Effects of salidroside (Sal) on the cell proliferation percentage of co-cultured RLE-6TN and NR 8383 cells

Sal (μg/mL)	Time (h)			
	0	6	12	24
0	(101.21 ± 4.98)%	(97.86 ± 5.34)%	(97.63 ± 5.29)%	(98.17 ± 3.57)%
2	(100.11 ± 3.32)%	(96.52 ± 6.49)%	(98.08 ± 4.61)%	(99.24 ± 5.38)%
8	(99.27 ± 4.09)%	(97.24 ± 4.73)%	(99.62 ± 4.16)%	(101.38 ± 4.67)%
32	(100.52 ± 4.15)%	(101.05 ± 5.35)%	(103.88 ± 4.54)%	(108.89 ± 3.23)%$^{*\#}$
128	(99.87 ± 5.28)%	(99.98± 4.56)%	(103.62 ± 5.52)%	(109.37 ± 4.28)%$^{*\#}$

Mean ± SD, $n = 8$. $^{*}P < 0.05$ *vs* 0 h group at the same dose level; $^{\#}P < 0.05$ *vs* 0 dose group at the same time level.

或 / 和 NR 8383 细胞 24 h 对细胞增殖百分率无显著影响 ($P > 0.05$，结果未展示)。

2.2 Sal刺激RLE-6TN细胞增殖与PI3K/AKT通路活化有关

不同剂量 Sal (0、2、8、32、128 μg/mL) 预处理 RLE-6TN 细胞 1 h 后继续培养 12 h，Western blot 检测细胞 p-AKT 和总 AKT 蛋白表达。与对照组 (非处理组) 相比，32 和 128 μg/mL Sal 预处理组 p-AKT/AKT 比值显著增加 ($P < 0.05$，图 1)。

2.3 Sal对LPS诱导RLE-6TN或/和NR 8383细胞分泌TNF-α的影响

Sal (32 μg/mL) 预处理 NR 8383 或 / 和 RLE-6TN 细胞 1 h 后，LPS (1 μg/mL) 刺激细胞 24 h，各组上清中 TNF-α 含量结果如图 2 所示：Sal 对 LPS 诱导 RLE-6TN 细胞分泌 TNF-α 无影响 ($P > 0.05$)，但能降低 LPS 诱导 NR 8383 细胞分泌 TNF-α ($P < 0.05$)；NR 8383 和 RLE-6TN 细胞共培养能降低 LPS 诱导 NR 8383 细胞分泌 TNF-α ($P < 0.05$)，而 Sal 能加强共培养的这一降低效应 ($P < 0.05$)。

2.4 Sal对LPS诱导RLE-6TN或/和NR 8383细胞分泌MIP-2的影响

如图 3 所示，Sal (32 μg/mL) 不仅能降低 LPS (1 μg/mL) 诱导 RLE-6TN 细胞分泌 MIP-2 ($P < 0.05$)，还能降低 LPS 诱导 NR 8383 细胞分泌 MIP-2 ($P < 0.05$)，NR 8383 和 RLE-6TN 细胞共培养能降低 LPS 诱导细胞分泌 MIP-2 ($P < 0.05$)，而 Sal 能加强共培养的这一降低效应 ($P < 0.05$)。

2.5 Sal对LPS诱导RLE-6TN或/和NR 8383细胞分泌IL-10的影响

如图 4 所示，Sal (32 μg/mL) 对 LPS (1 μg/mL) 诱导 RLE-6TN 细胞分泌 IL-10 无影响 ($P > 0.05$)，但增加 LPS 诱导 NR 8383 细胞分泌 IL-10 ($P > 0.05$)，NR 8383 和 RLE-6TN 细胞共培养能增加 LPS 诱导细胞分泌 IL-10 ($P < 0.05$)，而 Sal 能加强共培养的这一增加效应 ($P < 0.05$)。

3 讨论

红景天有效成分 Sal 的抗炎作用在动物和细胞

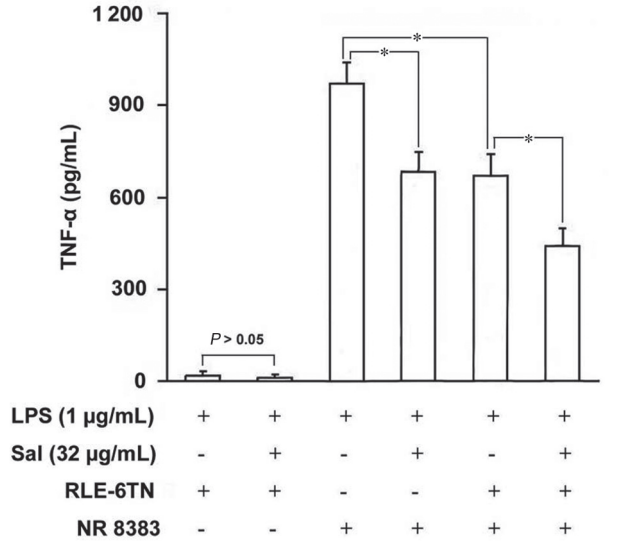

图 2. 红景天苷对脂多糖诱导RLE-6TN或/和NR 8383细胞分泌TNF-α的影响

Fig. 2. Effects of salidroside (Sal) on the secretion of TNF-α in lipopolysaccharide (LPS)-induced RLE-6TN or/and NR 8383 cells. RLE-6TN or NR 8383 cells were grown either alone or in co-culture with each other. Cells were treated with 1 μg/mL LPS for 24 h alone or with 32 μg/mL Sal pretreatment for 1 h. Enzyme linked immunosorbent assay (ELISA) was used to determine the content of TNF-α in supernatant. The data are expressed as the mean ± standard deviation of six independent experiments. $^{*}P < 0.05$.

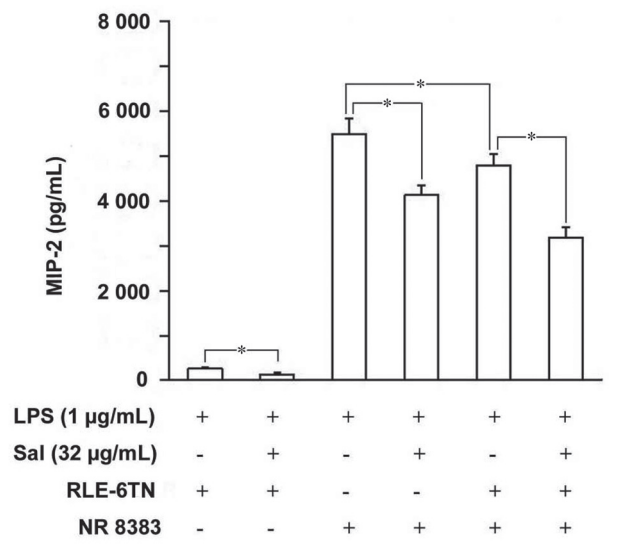

图 3. 红景天苷对脂多糖诱导RLE-6TN或/和NR 8383细胞分泌MIP-2的影响

Fig. 3. Effects of salidroside (Sal) on the secretion of MIP-2 in lipopolysaccharide (LPS)-induced RLE-6TN or/and NR 8383 cells. RLE-6TN or NR 8383 cells were grown either alone or in co-culture with each other. Cells were treated with 1 μg/mL LPS for 24 h alone or with 32 μg/mL Sal pretreatment for 1 h. Enzyme linked immunosorbent assay (ELISA) was used to determine the content of MIP-2 in supernatant. The data are expressed as the mean ± standard deviation of six independent experiments. $^{*}P < 0.05$.

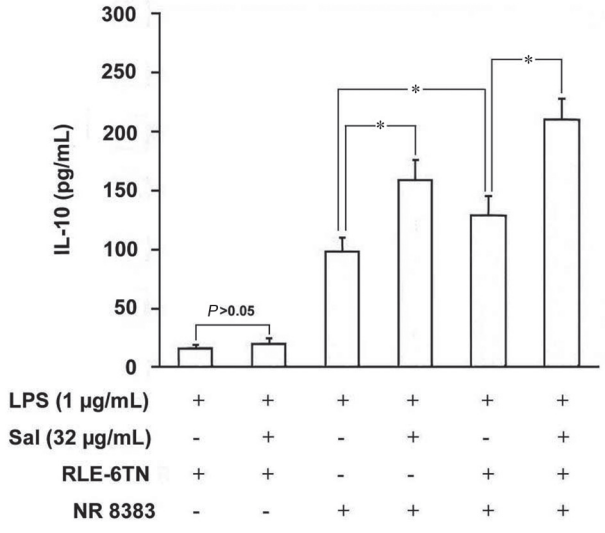

图 4. 红景天苷对脂多糖诱导RLE-6TN或/和NR 8383细胞分泌IL-10的影响

Fig. 4. Effects of salidroside (Sal) on the secretion of IL-10 in lipopolysaccharide (LPS)-induced RLE-6TN or/and NR 8383 cells. RLE-6TN or NR 8383 cells were grown either alone or in co-culture with each other. Cells were treated with 1 μg/mL LPS for 24 h alone or with 32 μg/mL Sal pretreatment for 1 h. Enzyme linked immunosorbent assay (ELISA) was used to determine the content of IL-10 in supernatant. The data are expressed as the mean ± standard deviation of six independent experiments. $^{*}P < 0.05$.

水平已得到初步证实[9, 10, 14, 15]。在 LPS 气道滴注诱导的动物 ALI 模型中，本研究组以及其他研究小组证实 Sal 能减轻 LPS 诱导的肺内炎性损伤[11, 12]，但其抗 ALI 的具体细胞机制尚不清楚。AEC 损伤是 ALI 肺病理损伤的细胞学基础，AEC 能否得到有效的修复被认为是治疗 ALI 的关键[16]。AEC II 被认为是肺泡上皮的干细胞，其自我更新机制在 ALI 损伤修复中具有重要意义[17]。AM 是肺内主要的居留性吞噬细胞，也是 LPS 的主要效应细胞，AM 激活后可分泌 TNF-α、IL-1β、IL-6 和 MIP-2 等多种促炎介质，以及 IL-10 和 IL-13 等抗炎介质[2]。TNF-α 是重要的前炎症因子，AM 分泌的 TNF-α 不仅能促进 AM 继续活化，还能启动多种炎症因子如 IL-1β 和 IL-6 的分泌，放大炎症反应[18]。IL-10 是一种重要的抗炎介质，能抑制促炎细胞因子如 TNF-α 和 IL-1β 等合成，还能促进其他抗炎分子表达，在调节促炎与抗炎平衡中发挥重要作用[19]。组织学上，AM 与 AEC 在肺内相互邻近，提示 AM 与 AEC 之间的相互作用可能调节 AM 免疫应答反应[20]。

新近的研究显示，Sal 能促进细胞增殖和分化，如骨髓间质干细胞和雪旺细胞，发挥对受损组织的修复作用[21, 22]。本研究结果表明，在 RLE-6TN (AEC II 细胞系) 和 NR 8383 (AM 细胞系) 共培养体系中，不同剂量 Sal 预处理，32 和 128 μg/mL 剂量能增加共培养细胞的增殖百分率，而这一剂量范围 Sal 分别预处理 RLE-6TN 或 NR 8383 细胞，仅增加 RLE-6TN 细胞的增殖百分率，对 NR 8383 细胞的增殖百分率并无影响。PI3K/AKT 信号转导通路是细胞存活的重要通路，参与细胞生长和增殖[23]。因此，我们进一步检测了不同剂量 Sal 预处理对 RLE-6TN 细胞 p-AKT 和总 AKT 蛋白表达的影响，结果显示，p-AKT 蛋白表达水平相应增高，说明 Sal 可能通过活化 PI3K/AKT 信号通路促进 AEC II 细胞的增殖。

有研究显示，AEC II 的存在能抑制 LPS 诱导 AM 分泌炎症介质如 IL-6[13, 24]。因此，我们进一步研究 Sal 对 LPS 诱导 RLE-6TN 或 / 和 NR 8383 细胞分泌促炎和抗炎因子的作用，结果显示：在单细胞培养中，32 μg/mL Sal 预处理能抑制 LPS 诱导 NR 8383 细胞分泌 TNF-α 和 MIP-2，这与我们之前在小鼠单核巨噬细胞系 J774.1 中观察的结果类似，其机制可能通过干扰 LPS/TLR4/NF-κB 信号通路降低炎性介质的过度分泌[25]，32 μg/mL Sal 预处理还能促进 LPS 诱导 NR 8383 细胞分泌 IL-10；在共培养体系中，RLE-6TN 和 NR 8383 细胞共培养抑制 LPS 诱导 NR 8383 细胞分泌 TNF-α 和 MIP-2，促进 LPS 诱导 NR 8383 细胞分泌 IL-10，32 μg/mL Sal 预处理能进一步加强以上共培养作用。

综上所述，Sal 不仅能直接抑制 LPS 诱导的 AM 炎性活化，还可能通过 PI3K/AKT 信号通路促进 AEC II 增殖，参与 AEC II 对 LPS 诱导 AM 炎性活化的调节作用。AEC II 这种接触性抑制作用是否是通过分泌可溶性因子诱导其对 AM 的调节作用值得进一步探讨。

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