

## M2巨噬细胞通过调控NF- $\kappa$ B信号通路对非小细胞肺癌A549细胞上皮-间质转化和顺铂耐药的促进作用

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**[摘要]** **目的:** 探讨M2巨噬细胞在非小细胞肺癌(NSCLC)上皮-间质转化(EMT)和顺铂(DDP)耐药中的作用, 阐明核因子 $\kappa$ B(NF- $\kappa$ B)信号通路的调控机制。**方法:** 选取人单核细胞白血病THP-1细胞, 通过佛波酯(PMA)诱导分化为M0巨噬细胞, 白细胞介素(IL)-4和IL-13联合诱导M0巨噬细胞分化为M2巨噬细胞。采用Western blotting法和免疫荧光法检测M0和M2巨噬细胞中CD163、CD86及精氨酸酶1(Arg-1)蛋白表达情况。选取人NSCLC细胞A549, 采用Transwell小室分别与M0和M2巨噬细胞非接触式共培养, 细胞分为A549+M0组(A549细胞与M0巨噬细胞共培养)、A549+M2组(A549细胞与M2巨噬细胞共培养)和A549+M2+BAY11-7082组(A549细胞与M2巨噬细胞共培养后加入10 mmol·L<sup>-1</sup> NF- $\kappa$ B抑制剂BAY11-7082)。细胞划痕实验检测各组A549细胞划痕愈合率, Transwell小室实验检测各组A549细胞中侵袭细胞数, 细胞计数试剂盒8(CCK-8)法检测共培养体系中DDP处理的各组A549细胞生长抑制率和半数抑制浓度(IC<sub>50</sub>)值, Western blotting法检测各组A549细胞中波形蛋白(Vimentin)、E钙黏蛋白(E-cadherin)、N钙黏蛋白(N-cadherin)、转录因子Snail、磷酸化P65(p-P65)、P糖蛋白(P-gp)和细胞程序性死亡配体1(PD-L1)蛋白表达水平。**结果:** Western blotting法, 与M0组比较, M2组巨噬细胞中CD163和Arg-1蛋白表达水平均明显升高( $P<0.05$ ), CD86蛋白表达水平明显降低( $P<0.05$ )。免疫荧光法, 与M0组比较, M2组巨噬细胞中CD163蛋白表达增强, CD86蛋白表达减弱。细胞划痕实验, 培养24和48 h时, 与A549+M0组比较, A549+M2组A549细胞划痕愈合率均明显升高( $P<0.05$ ); 3组共培养体系中, 与A549+M0组比较, A549+M2组A549细胞划痕愈合率明显升高( $P<0.05$ ); 与A549+M2组比较, A549+M2+BAY11-7082组A549细胞中划痕愈合率明显降低( $P<0.05$ )。Transwell小室实验, 与A549+M0组比较, A549+M2组A549细胞中侵袭细胞数明显增加( $P<0.05$ ); 与A549+M2组比较, A549+M2+BAY11-7082组A549细胞中侵袭细胞数明显减少( $P<0.05$ ); 3组共培养体系中, 与A549+M0组比较, A549+M2组A549细胞中侵袭细胞数明显增加( $P<0.05$ )。CCK-8法, 2.50、5.00、10.00、20.00和40.00 mg·L<sup>-1</sup> DDP处理后, 与A549+M0组比较, A549+M2组A549细胞生长抑制率均明显降低( $P<0.05$ 或 $P<0.01$ ), IC<sub>50</sub>值均明显升高( $P<0.01$ ); 3组共培养体系中, 与A549+M0组比较, A549+M2组A549细胞生长抑制率均明显降低( $P<0.05$ 或 $P<0.01$ ), IC<sub>50</sub>值均明显升高( $P<0.01$ ); 与A549+M2组比较, A549+M2+BAY11-7082组A549细胞生长抑制率明显升高( $P<0.05$ ), IC<sub>50</sub>值均明显降低( $P<0.05$ )。Western blotting法, 与A549+M0组比较, A549+M2组A549细胞中E-cadherin蛋白表达水平明显降低( $P<0.05$ ), N-cadherin、Vimentin和Snail蛋白表达水平均明显升高( $P<0.05$ ); 3组共培养体系中, 与A549+M0组比较, A549+M2组A549细胞中

[收稿日期] 2024-06-18 [录用日期] 2024-08-24

[基金项目] 吉林省卫健委卫生健康科技能力提升项目(2023JC033)

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E-cadherin蛋白表达水平明显降低 ( $P < 0.05$ ), N-cadherin、Snail、Vimentin和p-P65蛋白表达水平均明显升高 ( $P < 0.05$ ); 与A549+M2组比较, A549+M2+BAY11-7082组A549细胞中, E-cadherin蛋白表达水平明显升高 ( $P < 0.05$ ), Vimentin、N-cadherin和p-P65蛋白表达水平均明显降低 ( $P < 0.05$ )。与A549+M0组比较, A549+M2组A549细胞中P-gp和PD-L1蛋白表达水平均明显升高 ( $P < 0.05$ ); 3组共培养体系中, 与A549+M0组比较, A549+M2组A549细胞中P-gp和PD-L1蛋白表达水平均明显升高 ( $P < 0.05$ ); 与A549+M2组比较, A549+M2+BAY11-7082组A549细胞中P-gp和PD-L1蛋白表达水平均明显降低 ( $P < 0.05$ )。结论: M2巨噬细胞可调控NSCLC细胞EMT促进肿瘤侵袭转移, 调控P-gp和PD-L1蛋白表达促进DDP耐药, 其作用机制可能与NF- $\kappa$ B信号通路有关。

[关键词] 肿瘤相关巨噬细胞; 非小细胞肺癌; 核因子 $\kappa$ B; 上皮-间质转化; 顺铂

[中图分类号] R734.2 [文献标志码] A

## Promotive effect of M2 macrophages on epithelial-mesenchymal transition and cisplatin resistance in non-small cell lung cancer A549 cells by regulating NF- $\kappa$ B signaling pathway

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**ABSTRACT** **Objective:** To discuss the role of M2 macrophages in epithelial-mesenchymal transition (EMT) and cisplatin (DDP) resistance in the non-small cell lung cancer (NSCLC), and to clarify the regulatory mechanism of nuclear factor  $\kappa$ B (NF- $\kappa$ B) signaling pathway. **Methods:** The human monocytic leukemia THP-1 cells were selected and differentiated into M0 macrophages by phorbol myristate acetate (PMA) induction, followed by M2 macrophage polarization through interleukin (IL)-4 and IL-13 stimulation. Western blotting and immunofluorescence methods were used to detect the protein expression levels of CD163, CD86, and arginase-1 (Arg-1) in M0 and M2 macrophages. The human NSCLC A549 cells were co-cultured non-contactly with M0 or M2 macrophages in Transwell chambers, and the cells were divided into A549+M0 group (A549 cells co-cultured with M0 macrophages), A549+M2 group (A549 cells co-cultured with M2 macrophages), and A549+M2+BAY11-7082 group (A549 cells co-cultured with M2 macrophages and treated with  $10 \text{ mmol} \cdot \text{L}^{-1}$  NF- $\kappa$ B inhibitor BAY11-7082). Wound healing assay was used to detect the wound healing rate of the A549 cells in various groups; Transwell assay was used to detect the number of invasion A549 cells in various groups; cell counting kit-8 (CCK-8) assay was used to detect the inhibitory rate of proliferation and half maximal inhibitory concentration ( $\text{IC}_{50}$ ) value of the A549 cells after treated with DDP in the co-culture system; Western blotting method was used to detect the expression levels of vimentin, E-cadherin, N-cadherin, transcription factor Snail, phosphorylated P65 (p-P65), P-glycoprotein (P-gp), and programmed death-ligand 1 (PD-L1) proteins in the A549 cells in various groups. **Results:** The Western blotting results showed that compared with M0 group, the expression levels of CD163 and Arg-1 proteins in the macrophages in M2 group were significantly increased ( $P < 0.05$ ), while the expression level of CD86 protein was significantly decreased ( $P < 0.05$ ). The immunofluorescence results showed that compared with M0 group, the expression of CD163 protein in the macrophages in M2 group was enhanced and the expression of CD86 protein was weakened. The wound healing assay results showed that at 24 and 48 h of culture, compared with A549+M0 group, the wound healing rate of the A549 cells in A549+M2 group was significantly increased ( $P < 0.05$ ); in the co-culture system, compared with A549+M0 group, the wound healing rate

of the A549 cells in A549+M2 group was significantly increased ( $P<0.05$ ); compared with A549+M2 group, the wound healing rate of the A549 cells in A549+M2+BAY11-7082 group was significantly decreased ( $P<0.05$ ). The Transwell assay results showed that compared with A549+M0 group, the number of invasion A549 cells in A549+M2 group was significantly increased ( $P<0.05$ ); compared with A549+M2 group, the number of invasion A549 cells in A549+M2+BAY11-7082 group was significantly decreased ( $P<0.05$ ); in the co-culture system, compared with A549+M0 group, the number of invasion A549 cells in A549+M2 group was significantly increased ( $P<0.05$ ). The CCK-8 assay results showed that after treated with 2.50, 5.00, 10.00, 20.00, and 40.00  $\text{mg}\cdot\text{L}^{-1}$  DDP, compared with A549+M0 group, the inhibitory rate of proliferation of the A549 cells in A549+M2 group was significantly decreased ( $P<0.05$  or  $P<0.01$ ), and the  $\text{IC}_{50}$  value was significantly increased ( $P<0.01$ ); in the co-culture system, compared with A549+M0 group, the inhibitory rate of proliferation of the A549 cells in A549+M2 group was significantly decreased ( $P<0.05$  or  $P<0.01$ ), and the  $\text{IC}_{50}$  value was significantly increased ( $P<0.01$ ); compared with A549+M2 group, the inhibitory rate of proliferation of the A549 cells in A549+M2+BAY11-7082 group was significantly increased ( $P<0.05$ ), and the  $\text{IC}_{50}$  value was significantly decreased ( $P<0.05$ ). The Western blotting results showed that compared with A549+M0 group, the expression level of E-cadherin proteins in the A549 cells in A549+M2 group was significantly decreased ( $P<0.05$ ), while the expression levels of N-cadherin, vimentin, and Snail proteins were significantly increased ( $P<0.05$ ); in the co-culture system, compared with A549+M0 group, the expression levels of vimentin, Snail, N-cadherin, and p-P65 proteins in the A549 cells in A549+M2 group were significantly increased ( $P<0.05$ ), while the expression level of E-cadherin proteins was significantly decreased ( $P<0.05$ ); compared with A549+M2 group, the expression levels of vimentin, N-cadherin, and p-P65 proteins in the A549 cells in A549+M2+BAY11-7082 group were significantly decreased ( $P<0.05$ ), while the expression level of E-cadherin proteins was significantly increased ( $P<0.05$ ); compared with A549+M0 group, the expression levels of P-gp and PD-L1 proteins in the A549 cells in A549+M2 group were significantly increased ( $P<0.05$ ); in the co-culture system, compared with A549+M0 group, the expression levels of P-gp and PD-L1 proteins in the A549 cells in A549+M2 group were significantly increased ( $P<0.05$ ); compared with A549+M2 group, the expression levels of P-gp and PD-L1 proteins in the A549 cells in A549+M2+BAY11-7082 group were significantly decreased ( $P<0.05$ ). **Conclusion:** The M2 macrophages can regulate EMT in the NSCLC cells to promote the invasion and metastasis of tumor, and modulate the expressions of P-gp and PD-L1 to enhance DDP resistance, which is associated with the NF- $\kappa$ B signaling pathway.

**KEYWORDS** M2 macrophage; Non-small cell lung cancer; Nuclear factor- $\kappa$ B; Epithelial-mesenchymal transition; Cisplatin

肺癌是我国发病率和死亡率最高的恶性肿瘤,其5年生存率低于15%<sup>[1]</sup>。非小细胞肺癌(non-small cell lung cancer, NSCLC)约占肺癌的80%~85%<sup>[2]</sup>。多数患者在就诊时已是中晚期,肿瘤转移和化疗耐药是影响其预后的主要原因<sup>[3]</sup>。研究<sup>[4]</sup>显示:肺癌的恶性进展与肿瘤微环境(tumor micro-environment, TME)密切相关。肿瘤相关巨噬细胞是TME中最常见的免疫细胞类型,约占肿瘤浸润细胞的50%<sup>[5]</sup>。肿瘤相关巨噬细胞可以分化为经典激活的M1型和替代激活的M2型,M1巨噬细胞由Th1细胞因子干扰素 $\gamma$ (interferon- $\gamma$ ,

IFN- $\gamma$ )等诱导产生,发挥抗肿瘤作用;M2巨噬细胞由Th2细胞因子白细胞介素(interleukin, IL)-4等诱导产生,发挥促肿瘤生长作用<sup>[6]</sup>。研究<sup>[7-9]</sup>显示:TME中M2巨噬细胞的浸润与肺癌、肝癌和卵巢癌等肿瘤转移及不良预后有密切关联。核因子 $\kappa$ B(nuclear factor- $\kappa$ B, NF- $\kappa$ B)信号通路是细胞存活的重要通路,不仅调控正常细胞的生物学过程,在肿瘤的发生发展中也发挥重要作用<sup>[10]</sup>。NF- $\kappa$ B信号通路的异常活化可促进肺癌细胞增殖、侵袭和转移及血管生成和化疗耐药等<sup>[11]</sup>。目前,M2巨噬细胞是否可以调控NF- $\kappa$ B信号通路促进

NSCLC进展尚未见报道。本研究以人单核细胞白血病THP-1细胞诱导的M2巨噬细胞和人NSCLC A549细胞为研究对象,通过非接触式细胞共培养实验模拟TME,观察M2巨噬细胞是否通过NF- $\kappa$ B信号通路促进A549细胞上皮-间质转化(epithelial-mesenchymal transition, EMT)和顺铂(cisplatin, DDP)耐药,以期为M2巨噬细胞促进肿瘤进展提供新的理论依据。

## 1 材料与方法

**1.1 细胞、主要试剂和仪器** 人NSCLC A549细胞购自上海中桥新舟生物科技有限公司,人单核细胞白血病THP-1细胞系购自中国科学院上海细胞库。DMEM培养基和THP-1细胞专用培养基购自武汉普诺赛生命技术公司,胎牛血清(fetal bovine serum, FBS)购自内蒙古奥普赛生物科技有限公司,IL-13购自以色列ProSpec公司,BCA蛋白浓度检测试剂盒、RIPA裂解液、佛波酯(phorbol 12-myristate 13-acetate, PMA)、IL-4、NF- $\kappa$ B抑制剂BAY11-7082、细胞程序性死亡配体1(programmed cell death-ligand 1, PD-L1)、波形蛋白(Vimentin)、E钙黏蛋白(E-cadherin)、N钙黏蛋白(N-cadherin)、P65和转录因子Snail抗体均购自上海碧云天生物技术公司,GAPDH、磷酸化P65(phosphorylated P65, p-P65)、精氨酸酶1(arginase-1, Arg-1)和CD86抗体购自成都正能生物技术公司,CD163和P糖蛋白(P-glycoprotein, P-gp)抗体购自武汉爱博泰克生物技术公司,DDP购自江苏豪森药业有限公司,Transwell小室(孔径:0.4和8.0  $\mu$ m)购自美国Corning公司,细胞计数试剂盒8(cell counting kit-8, CCK-8)购自武汉赛唯尔生物科技有限公司,Matrigel胶购自美国BD公司,ECL发光显色剂购自苏州莫纳生物科技有限公司。多功能酶标仪(型号:Infinite M200 pro)购自瑞士Tecan公司,荧光显微镜(型号:TH4-200)购自日本奥林巴斯公司,化学发光成像系统(型号:ChemStudio SA)购自德国耶拿公司。

**1.2 THP-1细胞和A549细胞培养** THP-1细胞培养于THP-1专用培养基,A549细胞培养于含10% FBS和1%青-链霉素的DMEM培养液中,置于37  $^{\circ}$ C、5%CO<sub>2</sub>孵箱中培养。

### 1.3 THP-1细胞诱导分化为M0和M2巨噬细胞

选取对数生长期的THP-1细胞,按每孔 $1 \times 10^6$ 个细胞的密度铺于6孔细胞培养板中,加入 $70 \mu\text{g} \cdot \text{L}^{-1}$

PMA培养24 h,诱导分化为M0巨噬细胞。在M0巨噬细胞中加入 $40 \mu\text{g} \cdot \text{L}^{-1}$  IL-4和 $20 \mu\text{g} \cdot \text{L}^{-1}$  IL-13培养48 h,诱导分化为M2巨噬细胞,收集M2巨噬细胞,采用Western blotting法和免疫荧光法检测细胞中M2巨噬细胞标志物(CD163和Arg-1)及M1巨噬细胞标志物(CD86)表达情况。

### 1.4 非接触细胞共培养体系建立及细胞分组

选用孔径0.4  $\mu$ m Transwell小室建立非接触式共培养体系,取 $5 \times 10^5$ 个A549细胞置于下室, $4 \times 10^5$ 个M0和M2巨噬细胞分别置于上室。实验首先分为A549+M0组(A549细胞与M0巨噬细胞共培养)和A549+M2组(A549细胞与M2巨噬细胞共培养),分析M2巨噬细胞在A549细胞EMT和顺铂耐药中的作用。再次分为A549+M0组(A549细胞与M0巨噬细胞共培养)、A549+M2组(A549细胞与M2巨噬细胞共培养)和A549+M2+BAY11-7082组,分析阻断NF- $\kappa$ B信号通路后M2巨噬细胞在A549细胞EMT和顺铂耐药中的作用。A549+M2+BAY11-7082组(A549细胞与M2巨噬细胞共培养后加入 $10 \text{mmol} \cdot \text{L}^{-1}$  NF- $\kappa$ B抑制剂BAY11-7082)。

**1.5 Western blotting法检测巨噬细胞表型蛋白、共培养体系各组A549细胞中EMT相关蛋白以及PD-L1和P-gp蛋白表达水平** RIPA裂解液提取细胞总蛋白,BCA试剂盒测定细胞蛋白浓度。以每孔30  $\mu$ g的浓度进行10% SDS-PAGE电泳,通过湿转法将蛋白条带转移至PVDF膜上,封闭液室温封闭15 min,加入一抗CD163、CD86、Arg-1、N-cadherin、E-cadherin、Vimentin、Snail、PD-L1、P-gp、p-P65、P65和GAPDH(1:1 000),4  $^{\circ}$ C孵育过夜。次日,加入二抗(1:3 000)室温孵育50 min,ECL曝光、显色,Image J软件分析蛋白条带灰度值,以GAPDH为内参,计算目的蛋白表达水平。目的蛋白表达水平=目的蛋白条带灰度值/内参蛋白条带灰度值。

### 1.6 免疫荧光法检测M0和M2巨噬细胞中CD163及CD86蛋白表达情况

0.25%胰酶消化M0和M2巨噬细胞,于共聚焦小皿中进行爬片。次日4%多聚甲醛室温固定20 min,免疫染色通透液室温孵育10 min,加入5% BSA,37  $^{\circ}$ C封闭30 min后加入一抗CD163(1:100)和CD86(1:100),4  $^{\circ}$ C过夜。次日,加入Cy3荧光标记的二抗,37  $^{\circ}$ C避光孵育1 h,DAPI染核5 min,封片,荧光显微镜拍

摄并采集图像。以免疫荧光强度代表 CD163 和 CD86 蛋白表达情况。

**1.7 细胞划痕实验检测共培养体系中各组 A549 细胞划痕愈合率** 选用孔径 0.4  $\mu\text{m}$  Transwell 小室 12 孔细胞培养板共培养体系, 按每孔  $5 \times 10^5$  个 A549 细胞的密度接种于下室, 当细胞呈单层融合时, 用 200  $\mu\text{L}$  枪头垂直划痕。磷酸盐缓冲液 (phosphate buffer saline, PBS) 洗去脱落细胞,  $2 \times 10^5$  个 M0 和 M2 巨噬细胞分别置于上室, 分别于 0、24 和 48 h 时间段对划痕区进行拍照。Image J 软件测量划痕面积, 计算细胞划痕愈合率, 以细胞划痕愈合率代表与巨噬细胞共培养的 A549 细胞迁移能力。细胞划痕愈合率 = (0 h 划痕面积 - 24/48 h 划痕面积) / 0 h 划痕面积  $\times 100\%$ 。

**1.8 Transwell 小室实验检测共培养体系中各组 A549 细胞中侵袭细胞数** 0.25% 胰酶消化与巨噬细胞共培养 48 h 的各组 A549 细胞, 以每孔  $6 \times 10^4$  个细胞的密度接种于已包被 50  $\mu\text{L}$  基质胶的孔径 8.0  $\mu\text{m}$  Transwell 小室上室, 无血清 DMEM 培养, 小室下层加入 600  $\mu\text{L}$  含 5% FBS 的 DMEM 培养液。培养 20 h 取出小室, 4% 多聚甲醛固定 20 min, 结晶紫染色 10 min, 显微镜下随机选取 5 个视野进行拍照, Image J 软件计算侵袭细胞数, 以侵袭细胞数代表与巨噬细胞共培养的 A549 细胞侵袭能力。

**1.9 CCK-8 法检测共培养体系中 DDP 处理的各组 A549 细胞生长抑制率和半数抑制浓度 (half maximal inhibitory concentration,  $\text{IC}_{50}$ ) 值** 0.25% 胰酶消化

与巨噬细胞共培养 48 h 的各组 A549 细胞, 以每孔  $3.5 \times 10^3$  个细胞的密度接种于 96 孔细胞培养板中, 每组设 3 个复孔。分别加入 1.25~40.00  $\text{mg} \cdot \text{L}^{-1}$  DDP, 培养 48 h 后每孔加入 10  $\mu\text{L}$  CCK-8 溶液, 37  $^{\circ}\text{C}$  孵育 2 h。采用酶标仪于波长 450 nm 处测定各孔吸光度 (A) 值, 计算细胞生长抑制率, 采用 SPSS 18.0 统计软件中 Probit 回归模型计算  $\text{IC}_{50}$  值。细胞生长抑制率 = (对照组平均 A 值 - 给药组平均 A 值) / 对照组平均 A 值  $\times 100\%$ 。

**1.10 统计学分析** 采用 SPSS 18.0 统计软件进行统计学分析, GraphPad Prism 9.5 软件绘制图像。2 组巨噬细胞中 CD163、CD86 和 Arg-1 蛋白表达水平, 各组共培养 A549 细胞划痕愈合率、侵袭细胞数、生长抑制率和  $\text{IC}_{50}$  值以及细胞中 EMT 相关蛋白、p-P65、P-gp 和 PD-L1 蛋白表达水平均符合正态分布, 以  $\bar{x} \pm s$  表示, 多组间样本均数比较采用单因素方差分析, 组间样本均数两两比较采用 LSD-*t* 检验, 2 组间样本均数比较采用两独立样本 *t* 检验。以  $P < 0.05$  为差异有统计学意义。

## 2 结果

**2.1 M0 和 M2 巨噬细胞中 CD163、CD86 和 Arg-1 蛋白表达水平** Western blotting 法检测结果显示: 与 M0 组比较, M2 组巨噬细胞中 CD163 和 Arg-1 蛋白表达水平均明显升高 ( $P < 0.05$ ), CD86 蛋白表达水平明显降低 ( $P < 0.05$ )。见图 1。免疫荧光法检测结果显示: 与 M0 组比较, M2 组巨噬细胞中 CD163 蛋白表达增强, CD86 蛋白表达减弱。见图 2。

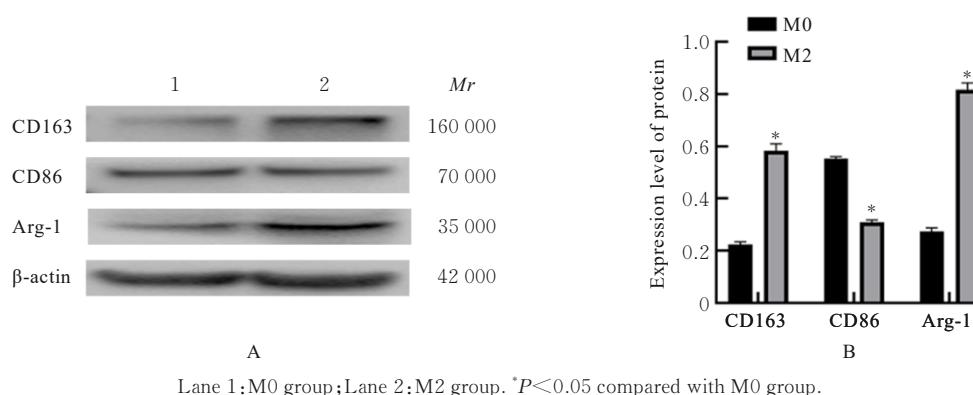


图 1 Western blotting 法检测 2 组巨噬细胞中 CD163、CD86 和 Arg-1 蛋白表达电泳图(A)及直条图(B)

Fig. 1 Electrophoregram(A) and histogram(B) of expressions of CD163, CD86, and Arg-1 proteins in macrophages in two groups detected by Western blotting method

**2.2 各组细胞划痕愈合率** 培养 24 和 48 h 时, 与 A549+M0 组比较, A549+M2 组 A549 细胞划痕

愈合率均明显升高 ( $P < 0.05$ )。见图 3 和表 1。3 组共培养体系中, 培养 24 和 48 h 时, 与 A549+M0 组

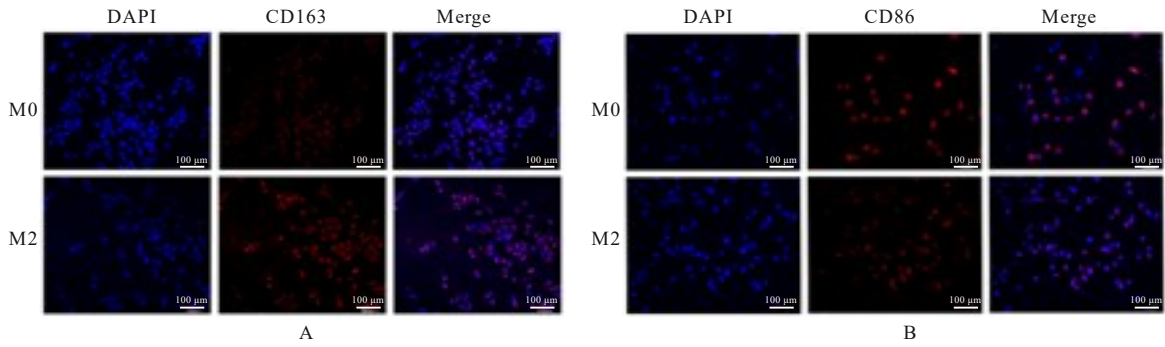


图2 免疫荧光法检测2组巨噬细胞中CD163(A)和CD86(B)蛋白表达情况

Fig. 2 Expressions of CD163(A) and CD86(B) proteins in macrophages in two groups detected by immunofluorescence method

比较, A549+M2组 A549细胞划痕愈合率均明显升高 ( $P<0.05$ ); 与 A549+M2组比较, A549+M2+BAY11-7082组 A549细胞划痕愈合率均明显降低 ( $P<0.05$ )。见图4和表2。

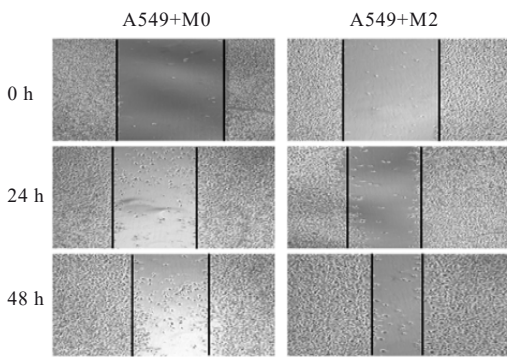


图3 2组A549细胞划痕愈合情况(×40)

Fig. 3 Scratch healing situations of A549 cells in two groups (×40)

表1 2组A549细胞划痕愈合率  
Tab. 1 Scratch healing rates of A549 cells in two groups  
( $n=3, \bar{x} \pm s, \eta/\%$ )

Group	Scratch healing rate	
	(t/h)	
A549+M0	21.83±2.80	28.26±2.68
A549+M2	29.02±3.17*	39.64±2.24*

\* $P<0.05$  compared with A549+M0 group.

2.3 各组A549细胞中侵袭细胞数 与A549+M0组(107.29个±10.24个)比较, A549+M2组A549细胞中侵袭细胞数(148.35个±14.85个)明显增加 ( $P<0.05$ )。3组共培养体系中, 与A549+M0组(98.73个±13.73个)比较,

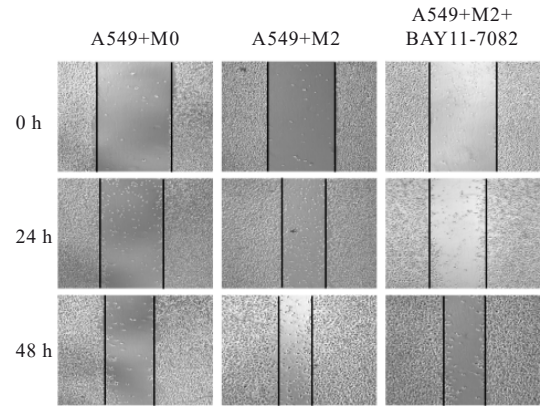


图4 3组共培养体系中A549细胞划痕愈合情况(×40)

Fig. 4 Scratch healing situations of A549 cells in co-culture system in three groups (×40)

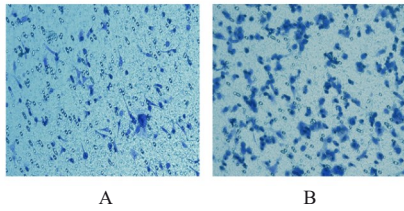
表2 3组共培养体系中A549细胞划痕愈合率  
Tab. 2 Scratch healing rates of A549 cells in co-culture system in three groups  
( $n=3, \bar{x} \pm s, \eta/\%$ )

Group	Scratch healing rate	
	(t/h)	
A549+M0	22.71±3.25	30.41±2.95
A549+M2	35.29±2.63*	45.02±2.41*
A549+M2+BAY11-7082	29.41±3.55 <sup>△</sup>	36.63±2.84 <sup>△</sup>

\* $P<0.05$  compared with A549+M0 group; <sup>△</sup> $P<0.05$  compared with A549+M2 group.

A549+M2组A549细胞侵袭细胞数(146.91个±18.48个)均明显增加 ( $P<0.05$ ); 与A549+M2组比较, A549+M2+BAY11-7082组A549细胞侵袭细胞数(108.65个±9.94个)明显减少 ( $P<0.05$ )。见图5和6。

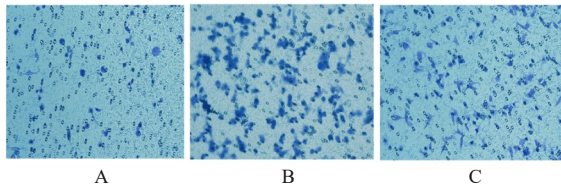
2.4 各组A549细胞生长抑制率和IC<sub>50</sub>值 2.50、5.00、10.00、20.00和40.00 mg·L<sup>-1</sup> DDP处理



A: A549+M0 group; B: A549+M2 group.

图5 2组A549细胞侵袭情况(结晶紫, ×200)

Fig. 5 Invasion of A549 cells in two groups (Crystal violet, ×200)



A: A549+M0 group; B: A549+M2 group; C: A549+M2+BAY11-7082 group.

图6 3组共培养体系中A549细胞侵袭情况(结晶紫, ×200)

Fig. 6 Invasion of A549 cells in co-culture system in three groups(Crystal violet, ×200)

后, 与A549+M0组比较, A549+M2组A549细胞生长抑制率均明显降低 ( $P<0.05$ 或 $P<0.01$ ),

$IC_{50}$ 值均明显升高 ( $P<0.01$ ), 1.25 mg·L<sup>-1</sup> DDP处理后, 2组A549细胞生长抑制率和 $IC_{50}$ 值比较差异均无统计学意义 ( $P>0.05$ )。3组共培养体系中, 与A549+M0组比较, A549+M2组A549细胞生长抑制率均明显降低 ( $P<0.05$ 或 $P<0.01$ ),  $IC_{50}$ 值均明显升高 ( $P<0.01$ ); 与A549+M2组比较, A549+M2+BAY11-7082组A549细胞生长抑制率明显升高 ( $P<0.05$ ),  $IC_{50}$ 值均明显降低 ( $P<0.05$ ), 1.25 mg·L<sup>-1</sup> DDP处理后, 各组A549细胞生长抑制率和 $IC_{50}$ 值比较差异均无统计学意义 ( $P>0.05$ )。见表3和4。

### 2.5 各组A549细胞中EMT相关蛋白和p-P65蛋白表达水平

与A549+M0组比较, A549+M2组A549细胞中E-cadherin蛋白表达水平明显降低 ( $P<0.05$ ), N-cadherin、Vimentin和Snail蛋白表达水平均明显升高 ( $P<0.05$ )。见图7。3组共培养体系中, 与A549+M0组比较, A549+M2组A549细胞中E-cadherin蛋白表达水平明显降低 ( $P<0.05$ ), Vimentin、Snail、N-cadherin和p-P65蛋白表达水平均明显升高 ( $P<0.05$ ); 与A549+M2组比较, A549+M2+BAY11-7082组A549细胞中E-cadherin蛋白表达水平明显升高 ( $P<0.05$ ), Vimentin、N-cadherin和p-P65蛋白表达水平均明显降低 ( $P<0.05$ )。见图8。

表3 不同浓度DDP处理后2组A549细胞生长抑制率和 $IC_{50}$ 值Tab. 3 Inhibitory rates of proliferation and  $IC_{50}$  values in A549 cells in two groups after treated with different concentrations of DDP ( $n=3, \bar{x} \pm s$ )

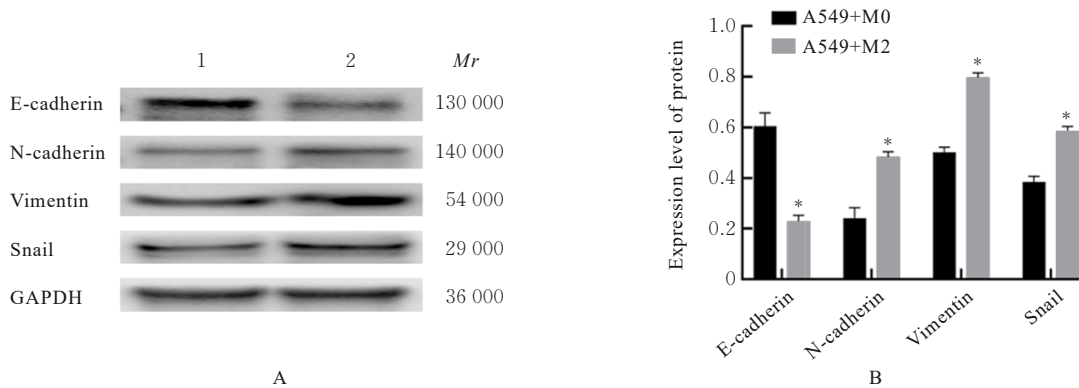
Group	Inhibitory rate of proliferation ( $\eta/\%$ )						$IC_{50}$ value [ $\rho_B/(\text{mg} \cdot \text{L}^{-1})$ ]
	1.25 mg·L <sup>-1</sup> DDP	2.50 mg·L <sup>-1</sup> DDP	5.00 mg·L <sup>-1</sup> DDP	10.00 mg·L <sup>-1</sup> DDP	20.00 mg·L <sup>-1</sup> DDP	40.00 mg·L <sup>-1</sup> DDP	
A549+M0	2.29±0.60	7.37±0.46	12.94±1.19	48.34±2.18	54.88±1.29	65.77±0.46	17.54±0.28
A549+M2	2.18±0.15	5.41±0.44*	9.40±1.47**	36.43±3.36**	45.31±3.11**	54.46±3.36**	26.55±2.94**

\* $P<0.05$ , \*\* $P<0.01$  compared with A549+M0 group.

表4 不同浓度DDP处理后3组共培养体系中A549细胞生长抑制率和 $IC_{50}$ 值Tab. 4 Inhibitory rates of proliferation and  $IC_{50}$  values of A549 cells in co-culture system in three groups after treated with different concentrations of DDP ( $n=3, \bar{x} \pm s$ )

Group	Inhibitory rate of proliferation ( $\eta/\%$ )						$IC_{50}$ value [ $\rho_B/(\text{mg} \cdot \text{L}^{-1})$ ]
	1.25 mg·L <sup>-1</sup> DDP	2.50 mg·L <sup>-1</sup> DDP	5.00 mg·L <sup>-1</sup> DDP	10.00 mg·L <sup>-1</sup> DDP	20.00 mg·L <sup>-1</sup> DDP	40.00 mg·L <sup>-1</sup> DDP	
A549+M0	2.25±0.23	7.35±0.41	15.20±0.75	44.68±1.85	56.50±1.33	62.95±2.98	18.21±0.79
A549+M2	2.07±0.16	4.17±1.89*	9.21±1.14**	33.76±2.09**	46.93±2.34**	53.07±1.55**	27.03±1.39**
A549+M2+BAY11-7082	2.19±0.19	6.43±0.78 <sup>△</sup>	13.82±2.20 <sup>△</sup>	40.50±1.64 <sup>△</sup>	53.07±1.52 <sup>△</sup>	56.85±1.39 <sup>△</sup>	21.80±0.71 <sup>△</sup>

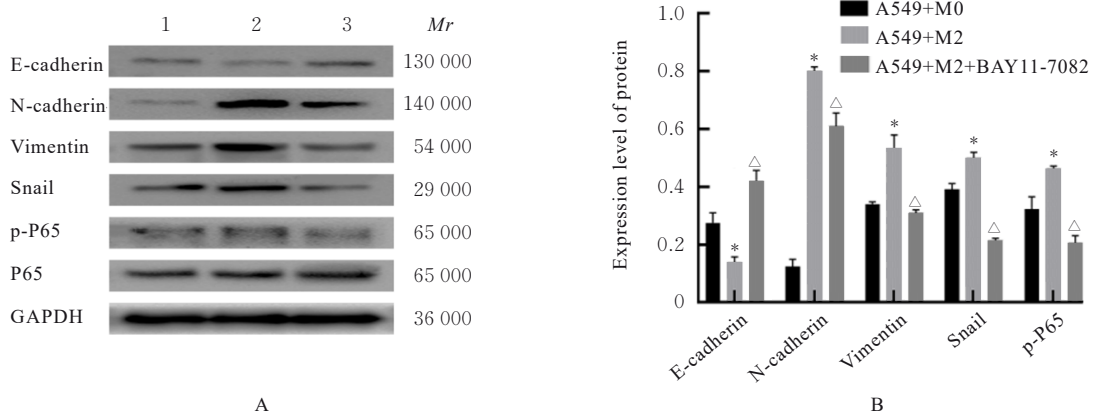
\* $P<0.05$ , \*\* $P<0.01$  compared with A549+M0 group; <sup>△</sup> $P<0.05$  compared with A549+M2 group.



Lane 1: A549+M0 group; Lane 2: A549+M2 group. \* $P < 0.05$  compared with A549+M0 group.

图7 Western blotting法检测2组A549细胞EMT相关蛋白电泳图(A)和直条图(B)

Fig. 7 Electrophoregram(A) and histogram(B) of expressions of EMT-related proteins in A549 cells in two groups detected by Western blotting method



Lane 1: A549+M0 group; Lane 2: A549+M2 group; Lane 3: A549+M2+BAY11-7082 group. \* $P < 0.05$  compared with A549+M0 group; <sup>△</sup> $P < 0.05$  compared with A549+M2 group.

图8 Western blotting法检测3组共培养体系中A549细胞中EMT相关蛋白表达电泳图(A)和直条图(B)

Fig. 8 Electrophoregram(A) and histogram(B) of expressions of EMT-related proteins in A549 cells in co-culture system in three groups detected by Western blotting method

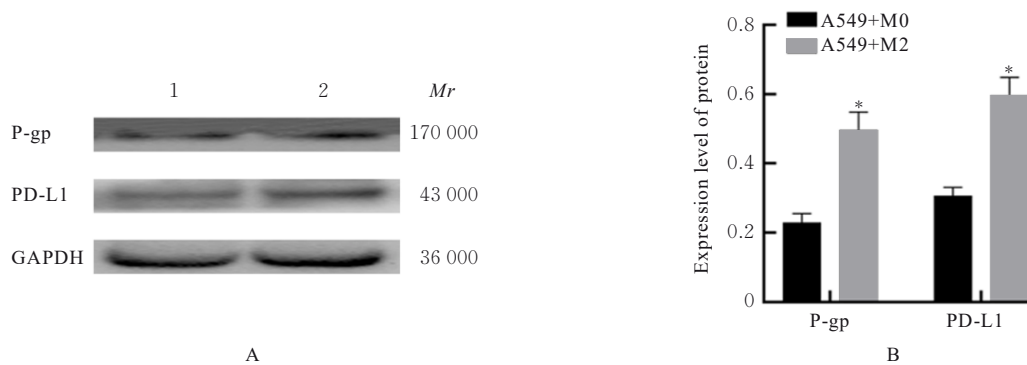
**2.6 各组A549细胞中P-gp和PD-L1蛋白表达水平**  
与A549+M0组比较, A549+M2组A549细胞中P-gp和PD-L1蛋白表达水平均明显升高 ( $P < 0.05$ )。见图9。3组共培养体系中, 与A549+M0组比较, A549+M2组A549细胞中P-gp和PD-L1蛋白表达水平均明显升高 ( $P < 0.05$ ); 与A549+M2组比较, A549+M2+BAY11-7082组A549细胞中P-gp和PD-L1蛋白表达水平均明显降低 ( $P < 0.05$ )。见图10。

### 3 讨论

研究<sup>[12]</sup>表明: 巨噬细胞可以诱导分化为M1和M2巨噬细胞, 其中M1巨噬细胞主要标志物为一氧化氮合酶 (inducible nitric oxide synthase,

iNOS) 和CD86, M2巨噬细胞主要标志物为CD206、CD163和Arg-1。本研究将THP-1细胞来源的M0巨噬细胞通过IL-4和IL-13诱导后, CD163和Arg-1蛋白表达明显上调, CD86蛋白表达明显下调, 提示M2巨噬细胞诱导成功。

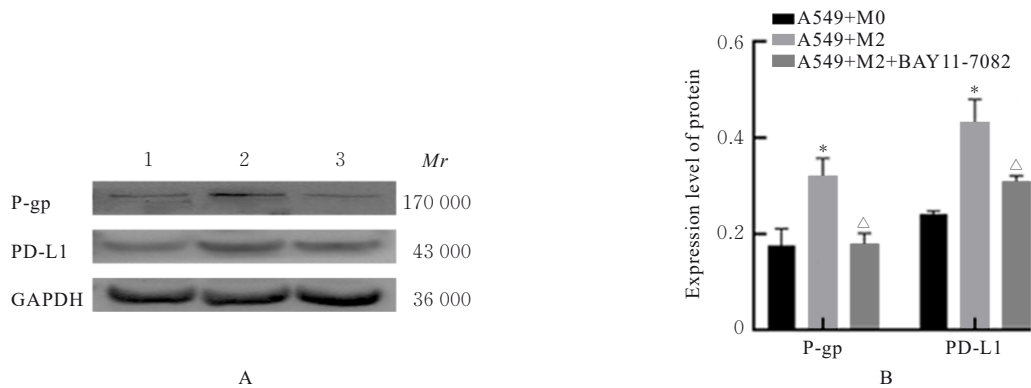
M2巨噬细胞在肿瘤细胞增殖转移、免疫抑制和化疗耐药等方面发挥重要作用<sup>[13-14]</sup>。研究<sup>[15]</sup>显示: TME中M2巨噬细胞与NSCLC淋巴结转移和肿瘤分期等恶性生物学行为有密切关联, 但其作用机制尚未完全阐明。EMT是肿瘤转移的关键步骤, 可通过上调Snail和扭曲螺旋转录因子(Twist), 诱导间质细胞标志物Vimentin和N-cadherin的生成, 抑制上皮细胞标志物E-cadherin表达, 进而增强肿瘤细胞的迁移和侵袭能力<sup>[16-17]</sup>。研究<sup>[18]</sup>显示: M2巨



Lane 1: A549+M0 group; Lane 2: A549+M2 group. \* $P < 0.05$  compared with A549+M0 group.

图9 Western blotting法检测2组A549细胞中P-gp和PD-L1蛋白表达电泳图(A)及直条图(B)

Fig. 9 Electrophoregram (A) and histogram (B) of expressions of P-gp and PD-L1 proteins in A549 cells in two groups detected by Western blotting method



Lane 1: A549+M0 group; Lane 2: A549+M2 group; Lane 3: A549+M2+BAY11-7082 group. \* $P < 0.05$  compared with A549+M0 group;  $\Delta P < 0.05$  compared with A549+M2 group.

图10 Western blotting法检测3组共培养体系中A549细胞中P-gp和PD-L1蛋白表达电泳图(A)及直条图(B)

Fig. 10 Electrophoregram (A) and histogram (B) of expressions of P-gp and PD-L1 proteins in A549 cells in co-culture system in three groups detected by Western blotting method

噬细胞可通过分泌IL-10细胞因子诱导肝癌和结肠癌等肿瘤细胞发生EMT进而促进肿瘤转移。本研究将THP-1细胞诱导的M2巨噬细胞与A549细胞共培养模拟肿瘤微环境,结果显示:与M2巨噬细胞共培养后A549细胞的迁移和侵袭能力明显增强,间质细胞表型和转录因子Snail蛋白表达上调,表明M2巨噬细胞可诱导NSCLC细胞发生EMT促进肿瘤侵袭转移。

肿瘤细胞高表达P-gp是化疗耐药的主要机制之一。P-gp是一种能量依赖性药物排出泵,可将化疗药物排出细胞外降低化疗效果<sup>[19]</sup>。研究<sup>[20]</sup>显示:M2巨噬细胞可通过分泌细胞因子[IL-10、转化生长因子 $\beta$ (transforming growth factor- $\beta$ , TGF- $\beta$ )和IL-6]激活肿瘤细胞P-gp产生耐药性。PD-L1表达在抗原提呈细胞和肿瘤细胞表面,通过与程序

性死亡受体1(programmed cell death ligand-1, PD-1)相互作用,抵消T淋巴细胞激活信号发挥免疫抑制功能<sup>[21]</sup>。研究<sup>[22]</sup>显示:肿瘤细胞PD-L1表达水平与化疗耐药密切相关。化疗耐药的NSCLC患者肿瘤组织中PD-L1蛋白表达水平明显升高,化疗后PD-L1蛋白表达上调与NSCLC患者不良预后有关<sup>[23]</sup>。目前,M2巨噬细胞对肺癌细胞P-gp和PD-L1蛋白的调控作用尚未见报道。本研究结果显示:与M2巨噬细胞共培养的A549细胞中P-gp和PD-L1蛋白表达上调,提示M2巨噬细胞可能通过调控NSCLC中P-gp和PD-L1蛋白的表达发挥化疗抵抗作用。DDP是NSCLC临床一线化疗药物,因此,本研究选用不同浓度DDP分别作用与M0和M2巨噬细胞共培养的A549细胞,结果显示:与M2巨噬细胞共培养后的A549细胞生长抑

制率降低, IC<sub>50</sub>值升高, 提示其对DDP的敏感性明显降低。以上结果表明: M2巨噬细胞可能通过调控P-gp和PD-L1蛋白的表达促进NSCLC细胞DDP耐药。

NF- $\kappa$ B不仅是重要的炎症通路, 还参与乳腺癌、脑癌和胃癌等多种肿瘤增殖、EMT、血管生成及化疗耐药过程<sup>[24]</sup>。研究<sup>[25-26]</sup>显示: M2巨噬细胞可激活结肠癌细胞NF- $\kappa$ B信号通路, 促进肿瘤的侵袭和转移, 还可通过分泌S100A9调控肝癌细胞NF- $\kappa$ B途径增强肿瘤干性。提示M2巨噬细胞诱导的NSCLC细胞EMT和DDP耐药可能与NF- $\kappa$ B信号通路有关。本研究结果与预期相符, A549细胞中NF- $\kappa$ B信号通路关键蛋白p-P65在与M2巨噬细胞共培养后表达水平明显升高, 表明M2巨噬细胞可激活A549细胞NF- $\kappa$ B信号通路。阻断A549细胞NF- $\kappa$ B信号通路可明显减弱M2巨噬细胞对A549细胞的促迁移和侵袭作用, 并逆转EMT相关蛋白的表达。此外, 抑制NF- $\kappa$ B信号通路, 可明显下调A549细胞P-gp和PD-L1蛋白表达, 并增加其对DDP的敏感性。以上结果表明: M2巨噬细胞可通过调控NF- $\kappa$ B信号通路促进NSCLC细胞EMT和DDP耐药, 但其通过何种机制活化NSCLC中NF- $\kappa$ B信号通路有待进一步深入研究。

综上所述, M2巨噬细胞可调控NSCLC细胞EMT促进肿瘤侵袭转移, 调控P-gp和PD-L1蛋白表达促进DDP耐药, 其作用机制与NF- $\kappa$ B信号通路有关。因此, 有效阻断M2巨噬细胞与NF- $\kappa$ B间的关联可能成为NSCLC免疫治疗的潜在靶点。

#### 利益冲突声明:

所有作者声明不存在利益冲突。

#### 作者贡献声明:

王星翔参与实验实施和论文撰写, 赵颖和任俏同参与部分实验操作、数据处理和统计学分析, 王鹤霏参与文献检索和数据整理, 蒲刚和历春参与研究指导及论文审校。

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