

芍药苷调控 JAK/STAT3 通路干预 HepG2 细胞 PD-L1 表达的研究

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摘要 为探究芍药苷对程序性死亡受体-配体 1 (programmed cell death-Ligand 1, PD-L1) 表达的影响及作用机制,研究采用干扰素 γ (IFN- γ) 诱导 HepG2 细胞建立体外 PD-L1 高表达细胞模型。采用 MTT 法检测芍药苷的细胞毒性,通过流式细胞术、ELISA、RT-PCR 法检测其对 PD-L1 蛋白和 mRNA 表达的影响;建立 HepG2 细胞和 Jurkat T 细胞共培养体系,ELISA 检测芍药苷干预后共培养 24 h 后的 IL-2 的表达,CCK-8 法检测药物干预后 T 细胞增殖情况,Western blot 检测芍药苷作用后 HepG2 细胞中 PD-L1、Janus 激酶 (Janus kinase, JAK) 和信号传导及转录激活因子 (signal transducers and activators of transcription, STAT3) 的蛋白表达。实验结果表明,芍药苷能够显著下调 PD-L1 蛋白和 mRNA 的表达水平,增加共培养体系中的 IL-2 的浓度,促进 T 细胞显著增殖,此外,芍药苷能显著抑制 JAK 和 STAT3 的蛋白磷酸化。实验结果表明,芍药苷能够下调 PD-L1 的表达,其机制可能与 JAK/STAT3 通路有关。

关键词 芍药苷;程序性死亡受体-配体 1;肿瘤免疫逃逸;免疫检查点

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Paeoniflorin inhibits programmed cell death-1-ligand 1 expression in HepG2 cells by regulating JAK/STAT3 signal pathway

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Abstract In order to explore the effect and its mechanism of paeoniflorin on PD-L1, a PD-L1 high expression cell model was established in interferon gamma (IFN- γ) -induced HepG2 cells. The cytotoxicity of paeoniflorin was detected by MTT assay. Flow cytometry, ELISA and RT-PCR were performed to detect protein and mRNA levels of PD-L1 regulated by paeoniflorin. In HepG2 cells and Jurkat T cell co-culture system, the expression of IL-2 was detected by ELISA. Besides, T cell proliferation was evaluated by CCK-8 method, and the protein expression levels of PD-L1, JAK and STAT3 after drug treatment were determined by Western blot. These results indicated that

paeoniflorin could significantly down-regulate the levels of PD-L1 protein and mRNA. In addition, it increased the number of T cells and the concentration of IL-2 in the co-culture system. Furthermore, paeoniflorin could significantly inhibit the protein expression of JAK and STAT3. All the above experimental data indicated that paeoniflorin could down-regulate the expression of PD-L1, and its mechanism might be related to the JAK/STAT3 pathway.

Key words paeoniflorin; PD-L1; tumor immune escape; immune checkpoint

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肝癌(hepatocellular carcinoma, HCC)是世界上最常见和最具侵袭性的肿瘤之一^[1-2]。尽管多激酶抑制剂在分子治疗方面取得了进展,但晚期HCC的预后仍然很差,5年生存率为3%~11%^[3]。免疫逃逸是肿瘤发展的重要因素,程序性死亡配体1(PD-L1)的过度表达参与肿瘤的免疫逃逸,与肿瘤的发生、发展及预后不良密切相关^[2,4]。PD-L1是PD-1的配体,在肿瘤细胞和免疫细胞上均表达,而PD-1主要在活化的T细胞上表达。PD-L1与PD-1的结合通过诱导T细胞的衰竭和凋亡来抑制T细胞效应功能,导致免疫抑制状态^[5]。研究表明PD-L1/PD-1信号通路在T细胞介导的肿瘤免疫应答中发挥重要作用,且针对PD-1/PD-L1的治疗性阻断已经证实可以使部分肿瘤患者具有持久的抗肿瘤反应^[6-7]。目前HCC的免疫治疗试验已发现组织学证实的肿瘤自发性消退,但患者反应率相对较低,并且基于抗体检查点抑制剂的不良反应和高成本限制了其应用^[8-9]。近年来,小分子抑制剂的研究逐步兴起,天然小分子对PD-L1调控的研究也取得了一定进展,挖掘潜在干扰PD-1/PD-L1表达的天然小分子可能是肿瘤治疗的新领域^[10-11]。

芍药苷(paeoniflorin)是一种单萜葡萄糖苷,作为天然植物药被广泛使用。芍药苷具有抗炎、镇痛、免疫调节、肝脏和神经保护、认知障碍改善和抗高血糖效应等药理作用^[12-13]。近年来临床前研究表明芍药苷对非小细胞肺癌、胃癌、肝癌和白血病等发挥抗肿瘤活性^[14-15],可通过调控PI3K/AK、JAK/STAT3、NF-κB等多种通路调节机体免疫功能,抑制肿瘤生长^[16-17]。因此,推测芍药苷可能通过JAK/STAT3通路调节PD-L1,参与肿瘤患者的免疫调控系统,增强抗肿瘤免疫应答。因此,本研究拟通过IFN-γ诱导的HepG2细胞模型探究芍药苷对PD-L1的调节作用及其作用机制。

1 材料

1.1 药品与试剂

芍药苷(HPLC面积归一化法测定纯度大于99%,四川维克奇生物科技有限公司);胎牛血清(美国Hyclone公司);DMEM、胰蛋白酶、噻唑兰MTT(江苏凯基生物技术股份有限公司);牛血清白蛋白(BSA)、IFN-γ(美国R&D公司);PD-L1 ELISA检测试剂盒(美国Protintech公司)、IL-2 ELISA检测试剂盒(美国Novus公司);PHA、PMA、Ruxolitinib(美国Sigma公司);BCA蛋白定量试剂盒(南京建成生物工程研究所);二甲基亚砜DMSO、RIPA裂解液、PMSF、Anti-PD-L1抗体(美国Cell Signaling Technology公司);Trizol(美国Life Technologies公司),引物、DEPC水(上海捷瑞生物工程有限公司);SYBR Green Real-time PCR Master Mix、ReverTra Ace qPCR RT Master Mix with gDNA Remover[东洋纺(上海)生物科技有限公司];其他试剂均为国产分析纯。

1.2 仪器

全波长多功能酶标仪(美国赛默飞世尔科技有限公司);超速控温离心机(美国Beckman Coulter公司);荧光倒置显微镜(德国Carl Zeiss公司);A101439电泳仪、QuantStudio™ Dx Real-Time PCR循环仪(美国Bio-Rad公司)。

1.3 细胞株

人肝癌细胞株HepG2购自上海ATCC细胞库。HepG2细胞用含10%FBS的高糖DMEM培养液,在37℃、体积分数5%CO₂的饱和湿度条件下培养,每隔3天用0.25%胰蛋白酶消化细胞,按1:3比例进行传代,取对数期的细胞用于实验^[18]。

2 方法

2.1 PD-L1高表达模型建立和药物溶解

IFN-γ 100 μg用去离子水500 μL溶解为

0.2 mg/mL母液,然后用PBS稀释为400 ng/mL储备液,0.22 μm滤膜过滤待用。

取一定量的芍药苷,先用DMSO溶解至一定浓度,使DMSO浓度不超过药物最终使用浓度的0.1%,然后用DMEM稀释至相应浓度。

2.1.1 ELISA检测IFN-γ对HepG2细胞PD-L1表达的影响 取对数生长期HepG2细胞,以每孔 1×10^6 个细胞接种于6孔板中,细胞贴壁后分别给予含有10、20、30 ng/mL IFN-γ的DMEM培养基,培养24 h后,裂解细胞后收集上清液,用ELISA试剂盒测定上清液中PD-L1的含量。同时抽提各孔细胞总蛋白,采用BCA定量试剂盒检测蛋白浓度,以校正PD-L1浓度。

2.1.2 Real-time PCR检测IFN-γ对HepG2细胞PD-L1表达的影响 HepG2细胞按照“2.1.1”项下方法处理后,PBS清洗细胞2次,用Trizol试剂提取细胞RNA,酶标仪测定 $A_{260/280}$ 及其浓度。RNA在65 °C条件下热变性5 min后,立即置于冰上,按照反转录试剂盒说明配制体系,使RNA反转录为cDNA,反应参数分别为:37 °C, 15 min; 50 °C, 5 min; 98 °C, 5 min。按照PCR试剂盒配制反应体系后进行反应,反应参数为95 °C, 15 s; 60 °C, 15 s; 72 °C, 45 s。其中,95 °C预变性60 s;共循环40次^[19]。测得基因引物序列见表1。

2.1.3 流式细胞术检测IFN-γ对HepG2细胞PD-L1表达的影响 HepG2细胞按照“2.1.1”项下方法处理后,用预冷的PBS清洗细胞两次,之后用胰酶消化收集离心后弃去上清液,细胞用0.1% BSA稀释的一抗稀释液(兔抗,1:400)100 μL重新悬浮,孵育1 h后,离心去上清液,用预冷PBS清洗两遍后,加入用0.1% BSA稀释的二抗稀释液(抗兔,1:8 000)100 μL重新悬浮,孵育40 min后,离心去上清液,用预冷PBS清洗两遍,加入PBS 50 μL重新悬浮后,用流式细胞术检测PD-L1蛋白的表达。

2.2 MTT法检测芍药苷对HepG2细胞的活性的影响

取生长状态良好的HepG2细胞,消化后以每孔 1×10^4 个细胞的密度接种于96孔板中,待细胞贴壁后吸去培养基,加入不含血清的DMEM饥饿8 h后,分别给予1,5,10,20,40 μmol/L的芍药苷,空白组加入不含血清的DMEM,孵育24 h后吸去培养基,每孔加入含5 mg/mL MTT的空白培养基

100 μL,孵育4 h,吸去上清液,每孔加入DMSO 150 μL,酶标仪测定490 nm处的吸收度,计算细胞活力,实验平行3次。

2.3 检测芍药苷对HepG2细胞PD-L1表达的影响

2.3.1 ELISA检测芍药苷对HepG2细胞PD-L1表达的影响 取对数生长期HepG2细胞,以每孔 1×10^6 个细胞接种于6孔板中,分别设置空白组(Control)、模型组(Model)和芍药苷给药组(20 μmol/L)。空白组每孔加入空白DMEM培养基,模型组每孔加入30 ng/mL IFN-γ的DMEM培养基,给药组每孔分别加入30 ng/mL IFN-γ和20 μmol/L芍药苷的DMEM培养基,孵育24 h后,裂解细胞后收集上清液,用ELISA试剂盒测定上清液中PD-L1的含量。同时抽提各孔细胞总蛋白,采用BCA定量试剂盒检测蛋白浓度,以校正PD-L1浓度。

2.3.2 Real-time PCR检测芍药苷对HepG2细胞PD-L1表达的影响 HepG2细胞按照“2.3.1”项下方法处理后,PBS清洗细胞2次,用Trizol试剂提取细胞RNA,酶标仪测定 $A_{260/280}$ 及其浓度。RNA在65 °C条件下热变性5 min后,立即置于冰上,按照反转录试剂盒说明配制体系,使RNA反转录为cDNA,反应参数分别为:37 °C, 15 min; 50 °C, 5 min; 98 °C, 5 min。按照PCR试剂盒配置反应体系后进行反应,反应参数为95 °C, 15 s; 60 °C, 15 s; 72 °C, 45 s。其中,95 °C预变性60 s;共循环40次。测得基因引物序列见表1。

Table 1 Primer sequence for real-time PCR assay

Gene	Primer sequence	
PD-L1	Forward	5'-GGTGCCGACTACAAGCGAAT-3'
	Reverse	5'-AGCCCTCAGCCTGACATGTC-3'
GAPDH	Forward	5'-ACAACTTGGTATCGTGGAGG-3'
	Reverse	5'-GCCATCACGCCACAGTTTC-3'

2.3.3 流式细胞术检测芍药苷对HepG2细胞PD-L1表达的影响 HepG2细胞按照“2.3.1”项下方法处理后,用预冷的PBS清洗细胞2次,之后用胰酶消化收集离心后弃去上清液,细胞用0.1% BSA稀释的一抗(1:400)100 μL重新悬浮,孵育1 h后,离心去上清液,用预冷PBS清洗两遍后,加入用0.1% BSA稀释的二抗(1:800)100 μL重新悬浮,孵育40 min后,离心去上清液,用预冷PBS清

洗两遍,加入 PBS 50 μ L 重新悬浮后,用流式细胞仪检测 PD-L1 蛋白的表达。

2.4 T 细胞和 HepG2 细胞共培养

取对数生长期 HepG2 细胞,以每孔 1×10^5 个接种于 12 孔板中,分别设置空白组(Control)、模型组(Model)和芍药苷给药组(20 μ mol/L)。给药后 1 h 加 IFN- γ ,孵育 24 h 后,用 PBS 清洗两遍后, Jurkat 细胞按照每孔 8×10^5 个细胞加入孔中,加入 10 μ g/mL PHA + 10 ng/mL PMA 孵育 24 h 后,收集上清液和 Jurkat 细胞,1 000 r/min 离心 5 min,分别检测 Jurkat 细胞活性和上清液中 IL-2 的浓度。

2.5 Western blot 分析

取对数生长期 HepG2 细胞,以每孔 1×10^6 个接种于 6 孔板中,分别设置空白组(Control)、模型组(Model)、芍药苷给药组(20 μ mol/L)和抑制剂组。空白组每孔加入空白 DMEM 培养基,模型组每孔加入 30 ng/mL IFN- γ 的 DMEM 培养基,给药组每孔分别加入 30 ng/mL IFN- γ 和 20 μ mol/L 芍药苷的 DMEM 培养基,孵育 24 h 后,PBS 清洗细胞两次,用细胞刮刮取细胞,收集后 300 r/min 离心 5 min,分别加蛋白裂解液 60 μ L 在冰上裂解 30 min,吹打细胞数次,收集裂解液,12 000 r/min 离心 15 min,收集上清液,用 BCA 试剂盒测定总蛋白浓度。将蛋白质样品稀释至相同且合适的浓度进行 SDS-PAGE 电泳(电泳参数:85 V、20 min;120 V、80 min),然后转移至 PVDF 膜上在 5% 脱脂奶粉封闭液中室温封闭 1 h,加入一抗(1:1 000)于 4 $^{\circ}$ C 过夜,后加入二抗(1:2 000)室温孵育 2 h,采用电化学发光检测法(ECL 法)显色。对感光胶片条带进行灰度分析,以目的条带与内参照条带 GAPDH 的比值代表目的蛋白的相对表达量。

2.6 数据分析

相关实验数据以 $\bar{x} \pm s$ 表示,用 SPSS 22.0 统计软件进行统计,多组间比较采用单因素方差分析。 $P < 0.05$ 为差异显著。

3 结果

3.1 HepG2 细胞 PD-L1 高表达模型的建立

IFN- γ 刺激 HepG2 细胞 24 h 后,ELISA 检测结果显示:30 ng/mL IFN- γ 对于 PD-L1 的上调作用最为明显(图 1),PCR 检测结果证明 IFN- γ 对于 PD-L1 mRNA 的上调呈现剂量依耐性(图 2),流式

细胞术检测结果也进一步证明了这个结论(图 3)。

3.2 芍药苷对 HepG2 细胞活力的影响

通过 MTT 检测不同浓度的芍药苷对 HepG2 细胞存活率的影响。如图 4 显示,与正常对照组相比,1、5、10、20 μ mol/L 的芍药苷处理后的细胞活力明显大于 90%,无明显细胞毒性,故选择 20 μ mol/L 芍药苷作为药物安全浓度开展后续实验。

3.3 芍药苷对 HepG2 细胞 PD-L1 表达的影响

如图 5 所示,与正常对照组相比,模型组细胞 PD-L1 的表达水平显著增加。加入芍药苷后,PD-L1 的表达受到抑制。ELISA 检测 PD-L1 与流式细胞术检测结果一致(图 6),30 ng/mL IFN- γ 处理的细胞 PD-L1 的蛋白和 mRNA 表达水平显著增加。与模型组相比,给予 20 μ mol/L 的芍药苷 24 h 后,给药组 HepG2 细胞的 PD-L1 蛋白和 mRNA 的表达水平显著降低(图 7)。

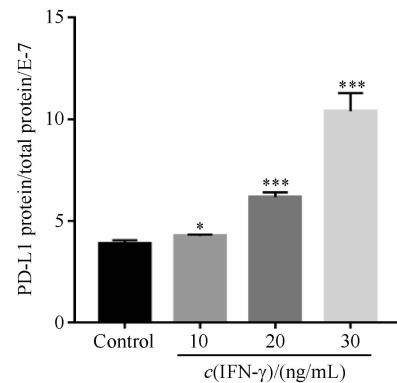


Figure 1 Effect of IFN- γ on PD-L1 expression in HepG2 cells. HepG2 cells were treated with different concentration of IFN- γ for 24 h, and then PD-L1 was measured by ELISA ($\bar{x} \pm s, n=4$)

* $P < 0.05$, *** $P < 0.001$ vs control group

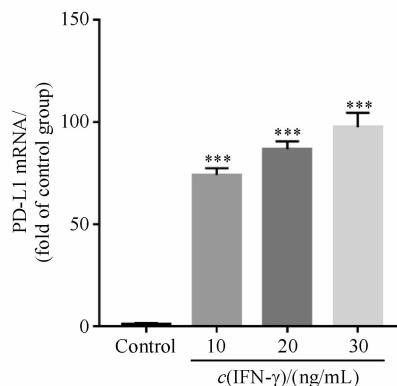


Figure 2 Effect of IFN- γ on PD-L1 mRNA expression of HepG2 cells. HepG2 cells were treated with different concentration of IFN- γ for 24 h, and then PD-L1 was measured by qPCR ($\bar{x} \pm s, n=3$)

*** $P < 0.001$ vs control group

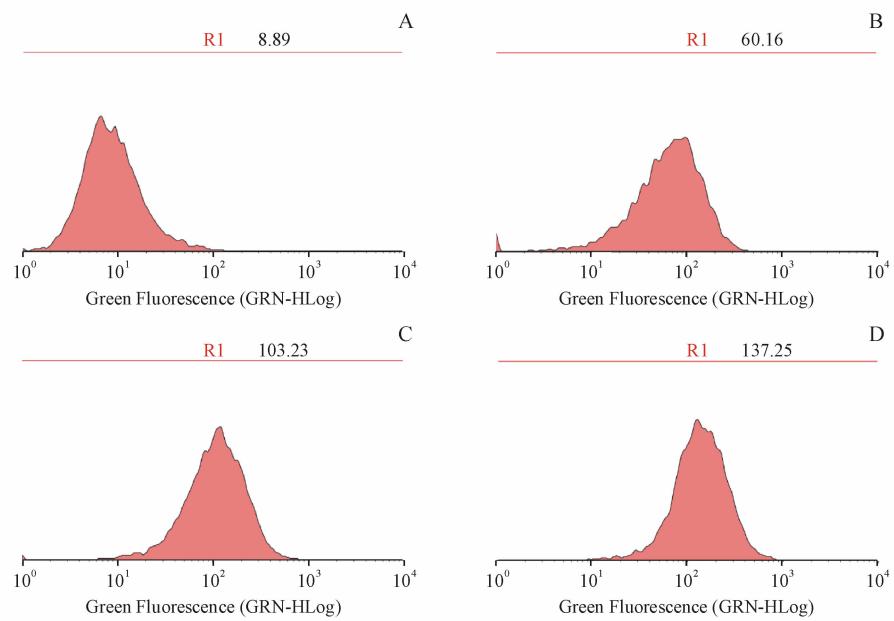


Figure 3 Effect of IFN- γ on PD-L1 protein expression of HepG2 cells. HepG2 cells were treated with different concentration of IFN- γ for 24 h, and then PD-L1 was measured by flow cytometry

A: Control group; B: HepG2 cell treated with 10 ng/mL IFN- γ ; C: HepG2 cell treated with 20 ng/mL IFN- γ ; D: HepG2 cell treated with 30 ng/mL IFN- γ

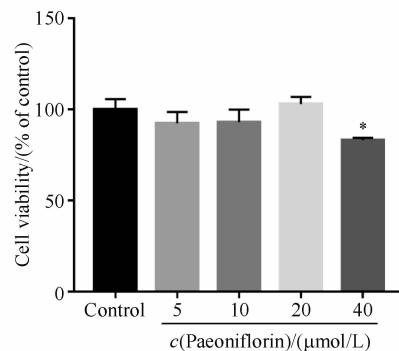


Figure 4 Effect of paeoniflorin on cell viability of HepG2 cells. HepG2 cells were treated with different concentration of paeoniflorin for 24 h, and then cell viability was measured by MTT assay ($\bar{x} \pm s, n=8$)

* $P < 0.05$ vs control group

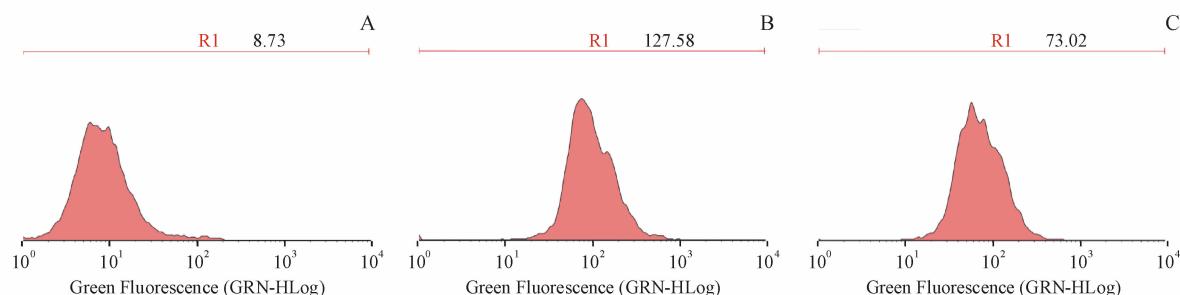


Figure 5 Effect of IFN- γ on PD-L1 protein expression of HepG2 cells. HepG2 cells were treated with different concentration of IFN- γ for 24 h, and then PD-L1 was measured by flow cytometry

A: Control group; B: HepG2 cell treated with 30 ng/mL IFN- γ ; C: HepG2 cell treated with 20 μ mol/L paeoniflorin in the absence or presence of 30 ng/mL IFN- γ for 24 h

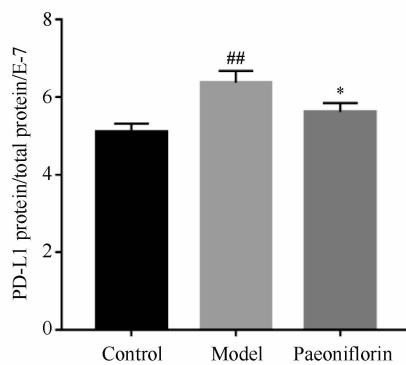


Figure 6 Effect of paeoniflorin on PD-L1 expression of HepG2 cells. HepG2 cells were treated with different concentration of IFN- γ for 24 h, and then PD-L1 concentration was measured by ELISA ($\bar{x} \pm s, n = 4$)

$^{##}P < 0.01$ vs control group; $^{*}P < 0.05$ vs model group

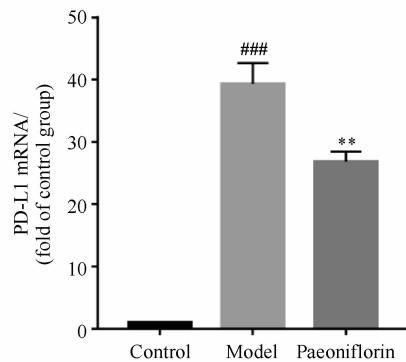


Figure 7 Effect of paeoniflorin on PD-L1 mRNA expression of HepG2 cells. HepG2 cells were treated with 20 $\mu\text{mol/L}$ paeoniflorin in the absence or presence of 30 ng/mL IFN- γ for 24 h, and then PD-L1 mRNA was measured by RT-PCR ($\bar{x} \pm s, n = 3$)

$^{###}P < 0.001$ vs control group; $^{**}P < 0.01$ vs model group

3.4 芍药苷对共培养体系中T细胞IL-2分泌的影响

为了检测芍药苷是否能够逆转PD-L1高表达的HepG2细胞对表达PD-1的T细胞的抑制作用,将IFN- γ 刺激的PD-L1高表达的HepG2细胞用芍药苷20 $\mu\text{mol/L}$ 处理24 h后,与激活的Jurkat T细胞共孵育,24 h后分别收集上清和Jurkat T细胞,检测IL-2的分泌水平和T细胞的增殖情况。如图8和9所示,模型组较空白组,HepG2高表达PD-L1,显著抑制T细胞的增殖活性,IL-2的分泌也受到抑制。加药组与模型组相比,芍药苷显著逆转了高表达PD-L1的HepG2细胞对高表达PD-1的T细胞的细胞增殖抑制作用,T细胞增殖活性显著增强,IL-2的分泌水平也显著提高。

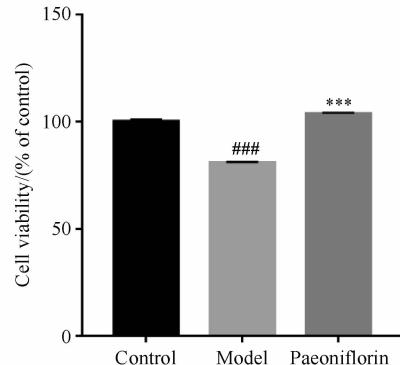


Figure 8 Effect of paeoniflorin on cell viability of Jurkat cells in co-culture system with HepG2 cells. HepG2 cells were treated with different concentration of IFN- γ for 24 h, and then the cell viability of Jurkat cells was measured by CCK-8 assay ($\bar{x} \pm s, n = 8$)

$^{###}P < 0.001$ vs control group; $^{***}P < 0.001$ vs model group

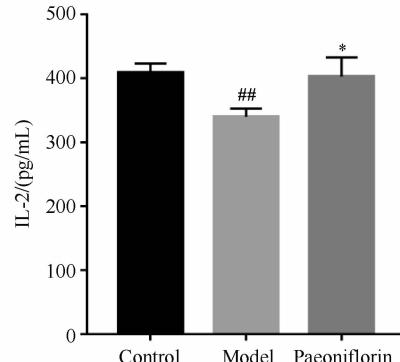


Figure 9 Effect of paeoniflorin on IL-2 secretion of Jurkat cells in co-culture system with HepG2 cells. HepG2 cells were treated with different concentration of IFN- γ for 24 h, and then IL-2 concentration was measured in cell-free supernatants using an ELISA kit ($\bar{x} \pm s, n = 4$)

$^{##}P < 0.01$ vs control group; $^{*}P < 0.05$ vs model group

3.5 芍药苷对HepG2细胞中PD-L1、JAK和STAT3磷酸化水平的影响

与正常组相比,IFN- γ 刺激下的模型组的PD-L1、JAK和STAT3的表达水平均显著上升。芍药苷干预后,PD-L1的表达显著降低,与抑制剂作用结果类似。JAK、STAT3的磷酸化水平在芍药苷作用后均有所降低(图10)。

4 讨论

肿瘤免疫逃逸是肿瘤发生发展的重要原因之一,PD-L1表达上调是肿瘤细胞的常见免疫逃避策略^[20],并预示着肿瘤治疗的预后不良。本研究采用IFN- γ 刺激HepG2细胞24 h,成功建立PD-L1高表达模型,评价天然小分子芍药苷对PD-L1的调控作用。实验结果证实芍药苷能够显著降低PD-L1

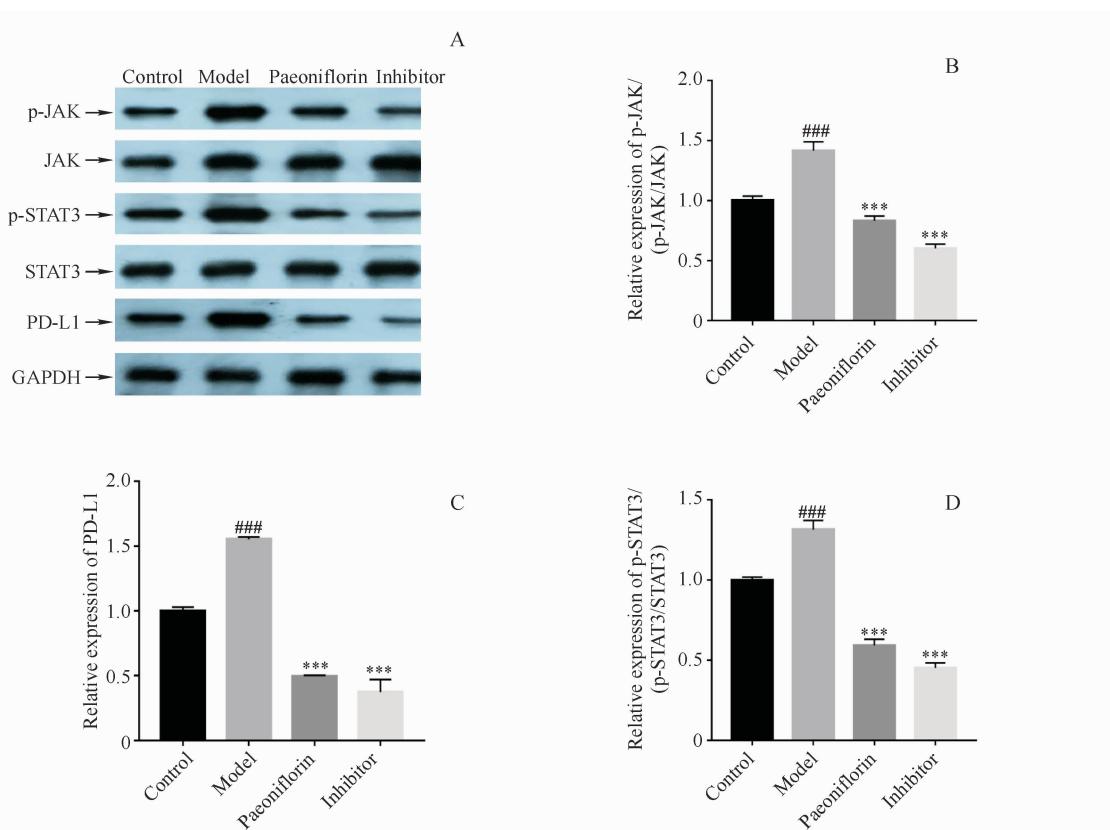


Figure 10 Effect of paeoniflorin on protein expression of JAK and STAT3 in HepG2 cells. Protein levels of PD-L1, JAK, p-JAK, STAT3, p-STAT3 were measured by Western blot in HepG2 cells treated with 20 μ mol/L paeoniflorin with or without IFN- γ in the absence or presence of JAK inhibitor INCB (A-D) ($\bar{x} \pm s, n=3$)

$P < 0.001$ vs control group; ** $P < 0.001$ vs model group

的表达,降低JAK、STAT3的磷酸化水平,提示芍药苷可能通过JAK/STAT3通路抑制PD-L1的表达,从而改善肿瘤免疫逃逸。

目前,靶向免疫检查点PD-1/PD-L1和CTLA-4的抗体治疗晚期肝细胞癌的临床试验正在进行中,PD-1/PD-L1抗体nivolumab在晚期HCC中的I/II期试验中显示出有利的结果,目前正在两项III期研究比较PD-1/PD-L1抗体和激酶抑制剂索拉非尼在HCC患者一二线治疗疗效,临床前研究数据明确了免疫检查点抑制剂在肿瘤治疗中的发展前景^[23],结果表明,抗PD-1抗体与局部治疗或其他靶向分子相结合药物是HCC的有效治疗策略。因此,免疫检查点抑制剂可以为HCC的治疗打开新的大门。但PD-1/PD-L1检查点抑制剂的高昂价格和所引起的严重免疫相关副反应限制了其在HCC中的应用。研究发现,天然小分子化合物如芹菜素、人参皂苷Rg3、雷公藤甲素、青藤碱等均能抑制PD-L1的表达,显示了天然小分子下调PD-L1表达的潜力。

已有研究表明,实体瘤可通过上调肿瘤微环境中的IFN- γ 诱导PD-L1表达,从而抑制抗肿瘤免疫应答^[5,24]。例如,肝细胞瘤细胞系(HepG2, Hep3B和PLC)暴露于IFN- γ 或IFN- α 后PD-L1的表达显著增加^[25]。本研究发现采用IFN- γ 刺激HepG2细胞,PD-L1蛋白和mRNA表达显著增加,说明模型成立。文献报道PD-1/PD-L1相互作用,引起T细胞凋亡和耗竭,抑制T细胞分泌IL-2,从而减弱T细胞对肿瘤细胞的抑制作用,促进肿瘤的发生发展^[26]。本研究结果显示,HepG2细胞经IFN- γ 刺激后高表达PD-L1,与活化的Jurkat细胞表达的PD-1结合,Jurkat细胞活性显著下降,其分泌的IL-2较空白组相比显著降低。加入芍药苷干预后,T细胞增殖显著增强,IL-2的分泌水平较模型组也显著增加。由此说明芍药苷能够通过抑制PD-L1的表达,从而逆转PD-L1与PD-1结合后对T细胞活性的抑制作用。

JAK/STAT3信号通路对免疫反应、细胞生长和分化等都发挥着至关重要的作用。细胞因子和

生长因子与激活非受体酪氨酸激酶 JAK 的膜受体结合,STAT 转录因子被活化的 JAK 磷酸化并二聚化,后入核与相应 DNA 片段结合,调控基因转录^[27]。STAT3 作为肿瘤发生过程中的癌蛋白已被广泛研究,其信号转导通路的不断激活与肿瘤的发生发展密切相关^[28-29]。JAK/STAT3 通路在多种癌症中被证明与 PD-L1 的表达调控密切相关,STAT3 能结合到 PD-L1 启动子区域从而调控 PD-L1 的表达^[30]。文献研究证实芍药苷可通过调控 STAT3 表达抑制人胶质瘤细胞、胃癌细胞等肿瘤细胞增殖^[16-17]。本研究发现芍药苷能显著降低 IFN-γ 诱导的 HepG2 细胞的 PD-L1 的高表达,降低细胞内 JAK、STAT3 的磷酸化水平,进一步提示芍药苷具有通过抑制 JAK/STAT3 信号通路达到抑制 PD-L1 表达的作用。

综上所述,芍药苷可能通过 JAK/STAT3 通路抑制 PD-L1 表达,从而发挥改善肿瘤免疫逃逸的作用,但仍需体内实验进一步验证。

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