

doi: 10.3978/j.issn.2095-6959.2018.11.006

View this article at: <http://dx.doi.org/10.3978/j.issn.2095-6959.2018.11.006>

慢病毒介导的 WWOX 表达对急性淋巴细胞白血病 细胞增殖活性的影响

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[摘要] 目的: 研究慢病毒介导的含WW域的氧化还原酶(WW Domain-containing Oxidoreductase, WWOX)表达对急性淋巴细胞白血病细胞增殖活性的影响。方法: 用过表达WWOX重组慢病毒和阴性对照重组慢病毒感染急性淋巴细胞白血病细胞Jurkat, 分别记为Lenti WWOX, Lenti NC; 用Realtime PCR和Western印迹法检测细胞中WWOX表达水平。用含有Wnt信号通路抑制剂FH535的培养液培养感染过表达WWOX重组慢病毒和感染阴性对照重组慢病毒的Jurkat细胞, 分别记为Lenti WWOX+FH535, Lenti NC+FH535; 用Realtime PCR和Western印迹法检测细胞中 β -catenin, c-myc表达水平。MTT测定细胞增殖活性, PI单染和Annexin V-FITC/PI双染法分别检测细胞周期和凋亡变化, Western印迹法检测细胞中周期相关蛋白Cyclin-D1, p27和凋亡蛋白C-Caspase-3表达水平。结果: Lenti WWOX细胞中WWOX表达水平明显高于Lenti NC($P < 0.05$)。Lenti WWOX, Lenti NC+FH535, Lenti WWOX+FH535细胞中 β -catenin, c-myc蛋白水平均明显低于Lenti NC, 并且Lenti WWOX + FH535细胞中 β -catenin, c-myc蛋白水平减少最多。Lenti WWOX, Lenti NC+FH535, Lenti WWOX+FH535细胞增殖能力降低, 细胞G₀/G₁比例升高, 细胞凋亡率升高, 细胞中Cyclin-D1蛋白水平降低, p27, C-Caspase-3蛋白水平升高, 与Lenti NC比较, 差异均有统计学意义($P < 0.05$)。Lenti WWOX+FH535细胞增殖能力降低, 细胞G₀/G₁比例升高, 细胞凋亡率升高, 细胞中Cyclin-D1蛋白水平降低, p27, C-Caspase-3蛋白水平升高, 与Lenti WWOX, Lenti NC+FH535比较, 差异均有统计学意义($P < 0.05$)。结论: 慢病毒介导的WWOX表达能够抑制急性淋巴细胞白血病细胞增殖, 阻滞细胞周期, 诱导细胞凋亡, 作用机制与抑制Wnt信号通路有关。

[关键词] 急性淋巴细胞白血病; 细胞周期; WWOX; Wnt信号通路

Effect of lentivirus mediated WWOX expression on proliferation of acute lymphoblastic leukemia cells

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Abstract **Objective:** To study the effect of lentivirus mediated WW Domain-containing Oxidoreductase (WWOX) expression on the proliferation of acute lymphoblastic leukemia. **Methods:** WWOX recombinant lentivirus and

收稿日期 (Date of reception): 2018-08-06

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infection negative control recombinant lentivirus infected acute lymphoblastic leukemia cells Jurkat were recorded as Lenti WWOX and Lenti NC, and WWOX expression levels were detected by Realtime PCR and Western blot methods. The Jurkat cells infected with WWOX recombinant lentivirus and negative control recombinant lentivirus were cultured with the culture solution containing the Wnt signaling pathway inhibitor FH535, and the Jurkat cells of the recombinant lentivirus and the negative control recombinant lentivirus were recorded as Lenti WWOX + FH535 and Lenti NC + FH535. The expression levels of β -catenin and c-myc were detected by Realtime PCR and Western blot. MTT assay cell proliferation activity, PI single staining and Annexin V-FITC/PI double staining were used to detect cell cycle and apoptosis respectively. The expression levels of Cyclin-D1, p27 and apoptotic protein C-Caspase-3 were detected by Western blot. **Results:** The expression level of WWOX in Lenti WWOX cells was significantly higher than that in Lenti NC ($P < 0.05$). The levels of β -catenin and c-myc protein in Lenti WWOX, Lenti WWOX + FH535 and Lenti NC + FH535 were significantly lower than those in Lenti NC. Moreover, the level of β -catenin and c-myc protein in Lenti WWOX + FH535 cells decreased most. The proliferation of Lenti WWOX, Lenti WWOX + FH535 and Lenti NC + FH535 were increased, the rate of apoptosis was increased, the level of Cyclin-D1 protein in the cells decreased, the levels of p27 and C-Caspase-3 protein were elevated, compared with Lenti NC, the difference was statistically significant ($P < 0.05$). The proliferation ability of Lenti WWOX + FH535 cells was reduced, the proportion of G_0/G_1 in cells increased, the rate of apoptosis was increased, the level of Cyclin-D1 protein in the cells decreased, the levels of p27 and C-Caspase-3 protein were elevated, compared with Lenti WWOX and Lenti NC + FH535, the difference was statistically significant ($P < 0.05$). **Conclusion:** The expression of lentivirus mediated WWOX can inhibit the proliferation of acute lymphoblastic leukemia cells, block cell cycle, and induce apoptosis, the mechanism of action is related to the inhibition of Wnt signaling pathway.

Keywords acute lymphoblastic leukemia; cell cycle; WWOX; Wnt signaling pathway

急性白血病是一种由造血干细胞恶性克隆引起的血液系统恶性肿瘤,分为急性淋巴性白血病和急性非淋巴性白血病,其中急性淋巴性白血病多发于儿童。研究急性淋巴性白血病分子发病机制是目前研究的重点^[1]。含WW域的氧化还原酶(WW Domain-containing Oxidoreductase, WWOX)是近年来发现的一种抑癌基因,其位于人类染色体脆性位点区域,在多种恶性肿瘤如乳腺癌、胃癌、胰腺癌等组织中表达下调,WWOX过表达可以诱导肿瘤细胞凋亡,抑制肿瘤细胞生长。最近的研究^[2-5]显示:WWOX在急性淋巴性白血病患者中表达水平降低,而对于其在白血病细胞增殖凋亡中的作用尚不清楚。Wnt信号通路是与肿瘤密切相关的细胞信号转导通路,其在肿瘤中过度激活,抑癌基因WWOX可以抑制Wnt信号通路的激活发挥抗肿瘤作用^[6]。本实验探讨WWOX在急性淋巴细胞白血病细胞增殖、细胞周期、凋亡中的作用及机制,为明确急性淋巴白血病发病机制提供参考。

1 材料与方法

1.1 材料

急性淋巴细胞白血病细胞Jurkat购自湖南丰晖生物有限公司, Jurkat细胞为悬浮生长细胞,细胞培养于含有10%胎牛血清的RPMJ 1640培养液中,细胞培养密度超过80%, 1 200 r/min离心10 min,添加新鲜的培养液,接种到细胞培养瓶内继续培养。Realtime PCR相关试剂购自美国Thermo公司; WWOX抗体、C-Caspase-3抗体购自美国Santa Cruz Biotech公司; 引物由南京金斯瑞生物科技有限公司合成; β -catenin抗体、c-myc抗体购自美国Abcam公司; 过表达WWOX重组慢病毒和阴性对照重组慢病毒购自上海锐赛生物技术有限公司; Cyclin-D1抗体、p27抗体购自碧云天生物技术研究; Wnt信号通路抑制剂FH535购自北京百奥莱博科技有限公司。

1.2 方法

1.2.1 慢病毒感染

Jurkat细胞接种到24孔板,每孔 2×10^4 个细胞

(200 μL 细胞悬浮液), 在细胞中添加慢病毒液, $\text{MOI}=30$, 继续培养72 h以后, 在荧光显微镜下观察感染效率高于90%。将感染过表达WWOX重组慢病毒和感染阴性对照重组慢病毒的Jurkat细胞记为Lenti WWOX, Lenti NC, 根据1.2.2和1.2.3中方法检测WWOX表达变化。

1.2.2 Realtime PCR 检测 WWOX 表达水平

收集Lenti WWOX, Lenti NC细胞, 以TRIzol试剂提取总RNA(步骤按照试剂盒标准流程操作), RNA经紫外分光光度计检测其 A_{260}/A_{280} 比值为1.8~2.0, 满足实验要求。配制反转录反应体系(包括6 μL RNA, 2 μL 5 \times RT Buffer, 1 μL Nuclear-free Water, 0.5 μL RT Enzyme Mix, 0.5 μL Primer Mix), 设置反转录条件为: 37 $^{\circ}\text{C}$ 15 min, 98 $^{\circ}\text{C}$ 5min, 保存在-20 $^{\circ}\text{C}$ 。引物序列为: β -actin F 5'-GGACCTGACTGACTACCTC-3', R 5'-TACTCCTGCTTGCTGAT-3'。WWOX F 5'-CACGCATTTTAGAAGAATGG-3', R 5'-GACAGCAGCACAGTACACG-3'。配制Realtime PCR反应体系(包括10 μL SYBR Green Realtime PCR Master Mix, 0.4 μL 的上下游引物, 5 μL 的cDNA), PCR反应程序设置为: 95 $^{\circ}\text{C}$ 30 s, 95 $^{\circ}\text{C}$ 5 s, 55 $^{\circ}\text{C}$ 5 s; 72 $^{\circ}\text{C}$ 15 s, 40个循环。根据每个反应的Ct值计算WWOX表达水平, 以管家基因 β -actin作为内参。

1.2.3 Western 印迹法检测 WWOX 表达水平

收集Lenti WWOX, Lenti NC细胞, 按照试剂盒标准步骤提取各组细胞总蛋白。提取蛋白样品经BCA法检测浓度后, 保存在-80 $^{\circ}\text{C}$ 。在每个泳道中添加40 μg 蛋白样品进行SDS-PAGE凝胶电泳(凝胶为12%分离胶、5%积层胶)。蛋白在上样前需变性处理(同1:1体积的2 \times Loading Buffer混合煮沸5 min)。设置在积层胶中以90 V的电压进行电泳(约30 min), 在分离胶中电压设置为120 V(电泳时间约为1.5 h)。将凝胶取出后, 常规方法转膜(转膜温度为4 $^{\circ}\text{C}$, 转膜电压为90 V, 转膜时长为2 h)、封闭(同5%牛血清白蛋白封闭液在室温条件下孵育1.5 h)。取PVDF膜, 放在1:600稀释的一抗在4 $^{\circ}\text{C}$ 过夜后, 再放在1:2 000稀释的二抗中, 在室温反应2 h。ECL试剂盒发光, 图像分析用Gel-Pro analyzer软件, 内参为 β -actin。

1.2.4 细胞分组处理

将感染过表达WWOX重组慢病毒和感染阴性对照重组慢病毒的Jurkat细胞分别用含有Wnt信号通路抑制剂FH535(终浓度为10 $\mu\text{mol/L}$)的细胞培养液培养, 分别记为Lenti WWOX + FH535, Lenti NC + FH535, Lenti NC, Lenti WWOX, Lenti NC +

FH535, Lenti WWOX + FH535细胞培养48 h以后, 用Realtime PCR和Western印迹法检测细胞中Wnt信号通路关键蛋白 β -catenin, c-myc表达水平, 步骤同1.2.3。

1.2.5 MTT 测定细胞增殖

Lenti NC, Lenti WWOX, Lenti NC + FH535, Lenti WWOX + FH535细胞分别接种到96孔板内, 按照上述处理方法分组以后, 培养至48 h时, 将培养板取出, 添加10 μL 的MTT, 孵育4 h, 弃上清, 再添加150 μL 的DMSO, 测定每孔570 nm的A值, 用不含细胞的空白孔调零, 将Lenti NC细胞存活率记为100%, 分析Lenti WWOX, Lenti NC + FH535, Lenti WWOX + FH535细胞存活率变化。

1.2.6 PI 单染法检测细胞周期

Lenti NC, Lenti WWOX, Lenti NC + FH535, Lenti WWOX + FH535细胞按照上述方法培养48 h以后, 每组收集 10^6 个细胞, 以冰预冷的PBS洗涤3次以后, 用70%的酒精固定, 在-20 $^{\circ}\text{C}$ 放置24 h。添加PI染液后, 用流式细胞仪检测细胞周期分布情况。

1.2.7 Annexin V-FITC/PI 双染法检测细胞凋亡

Lenti NC, Lenti WWOX, Lenti NC + FH535, Lenti WWOX + FH535细胞按照上述方法培养48 h以后, 每组收集 10^6 个细胞, 用500 μL 的结合缓冲液悬浮细胞, 添加PI染液和Annexin V-FITC染液, 用流式细胞仪检测细胞凋亡情况。

1.2.8 Western 印迹法检测细胞中周期蛋白 Cyclin-D1, p27 和凋亡蛋白 C-Caspase-3 表达水平

Lenti NC, Lenti WWOX, Lenti NC + FH535, Lenti WWOX + FH535细胞按照上述方法培养48 h以后, 收集细胞, 用Western印迹法检测细胞中周期蛋白Cyclin-D1, p27和凋亡蛋白C-Caspase-3表达水平, 步骤同1.2.3。

1.3 统计学处理

采用SPSS 21.0软件进行分析, 计量资料用均数 \pm 标准差($\bar{x}\pm s$)表示, 两组数据采用独立样本 t 检验, 多组差异比较用单因素方差分析, 组间比较采用SNK- q 检验, $P<0.05$ 为差异有统计学意义。

2 结果

2.1 过表达 WWOX 重组慢病毒提高 Jurkat 细胞中 WWOX 表达水平

过表达WWOX重组慢病毒感染后的Jurkat细胞中WWOX表达水平明显升高, 成功构建了过表达WWOX的Jurkat细胞株(图1, 表1)。

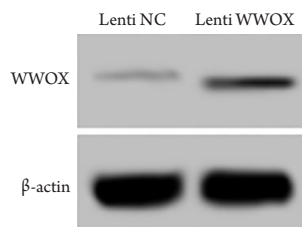


图1 Western印迹法检测过表达WWOX重组慢病毒对细胞中WWOX蛋白表达影响

Figure 1 Western blot detects the effect of recombinant WWOX Lentivirus on the expression of WWOX protein in cells

表1 过表达WWOX重组慢病毒感染后细胞中WWOX mRNA和蛋白表达水平($\bar{x} \pm s$)

Table 1 Expression of WWOX mRNA and protein in WWOX cells after overexpression of lentivirus infection ($\bar{x} \pm s$)

组别	WWOX mRNA	WWOX 蛋白
Lenti NC	1.00	0.10 ± 0.06
Lenti WWOX	2.35 ± 0.47	0.26 ± 0.03
<i>t</i>	4.975	4.131
<i>P</i>	<0.05	<0.05

2.2 过表达 WWOX 抑制 Jurkat 细胞中 Wnt 信号通路关键蛋白 β -catenin, c-myc 表达

过表达WWOX的Jurkat细胞中 β -catenin, c-myc表达水平明显降低, 过表达WWOX能够抑制Jurkat细胞中Wnt信号通路的激活。Wnt信号通路抑制剂FH535处理不仅可以下调Jurkat细胞中 β -catenin, c-myc表达水平, 还能够降低过表达WWOX的Jurkat细胞中 β -catenin, c-myc表达水平, Wnt信号通路抑制剂FH535能够抑制Jurkat细胞中Wnt信号通路激活(图2, 表2)。

2.3 Wnt 信号通路抑制剂协同 WWOX 抑制 Jurkat 细胞增殖

过表达WWOX和Wnt信号通路抑制剂FH535处理后的Jurkat细胞存活率明显降低, 并且二者联合后细胞存活率下降更多, Wnt信号通路抑制剂协同WWOX抑制Jurkat细胞增殖(表3)。

2.4 Wnt 信号通路抑制剂协同 WWOX 阻滞 Jurkat 细胞周期

过表达WWOX和Wnt信号通路抑制剂FH535处理后的Jurkat细胞G₀/G₁期比例明显升高, 细胞中

Cyclin-D1蛋白水平降低, p27蛋白水平升高, 并且二者联合对细胞周期阻滞作用更强, Wnt信号通路抑制剂协同WWOX阻滞Jurkat细胞周期(图3, 表4)。

2.5 Wnt 信号通路抑制剂协同 WWOX 诱导 Jurkat 细胞凋亡

过表达WWOX和Wnt信号通路抑制剂FH535处理后的Jurkat细胞凋亡率明显升高, 细胞中C-Caspase-3蛋白水平升高, 并且二者联合对细胞凋亡促进作用更强, Wnt信号通路抑制剂协同WWOX诱导Jurkat细胞凋亡(表5, 图4)。

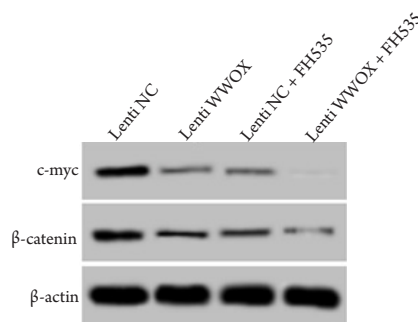


图2 过表达WWOX和Wnt信号通路抑制剂FH535对细胞中Wnt信号通路关键蛋白 β -catenin, c-myc表达影响

Figure 2 Effects of FH535, an inhibitor of WWOX and Wnt signaling pathways, on the expression of key Wnt signaling proteins β -catenin and c-myc in cells

表2 过表达WWOX及Wnt信号通路抑制剂FH535处理后细胞中Wnt信号通路关键蛋白 β -catenin, c-myc表达水平($\bar{x} \pm s$)

Table 2 Expression of Wnt signaling pathway key proteins β -catenin and c-myc in cells treated with WWOX and Wnt signaling pathway inhibitor FH535 ($\bar{x} \pm s$)

组别	β -catenin	c-myc
Lenti NC	0.62 ± 0.08	0.57 ± 0.04
Lenti WWOX	0.39 ± 0.05*	0.29 ± 0.03*
Lenti NC+FH535	0.34 ± 0.08*	0.25 ± 0.06*
Lenti WWOX+FH535	0.22 ± 0.02**&	0.07 ± 0.03**&
<i>F</i>	21.471	73.314
<i>P</i>	<0.001	<0.001

与 Lenti NC 相比, **P*<0.05; 与 Lenti WWOX 相比, **P*<0.05; 与 Lenti NC+FH535 比, &*P*<0.05。

Compared with Lenti NC, **P*<0.05; compared with Lenti WWOX, **P*<0.05; compared with Lenti NC+FH535, &*P*<0.05.

表3 Wnt信号通路抑制剂FH535对过表达WWOX的细胞存活率影响($\bar{x} \pm s$)

Table 3 Effect of Wnt signal pathway inhibitor FH535 on cell survival rate of overexpressing WWOX ($\bar{x} \pm s$)

组别	存活率/%
Lenti NC	100.00
Lenti WWOX	68.52 ± 7.32*
Lenti NC+FH535	64.01 ± 5.08*
Lenti WWOX+FH535	48.76 ± 7.94* [‡]
F	39.002
P	<0.001

与Lenti NC相比, * $P < 0.05$; 与Lenti WWOX相比, * $P < 0.05$; 与Lenti NC+FH535比, [‡] $P < 0.05$ 。

Compared with Lenti NC, * $P < 0.05$; compared with Lenti WWOX, * $P < 0.05$; compared with Lenti NC+FH535, [‡] $P < 0.05$.

表4 Wnt信号通路抑制剂FH535对过表达WWOX的细胞周期分布及细胞中Cyclin-D1, p27蛋白表达影响($\bar{x} \pm s$)

Table 4 Effects of FH535, an inhibitor of Wnt signaling pathway, on cell cycle distribution and expression of Cyclin-D1 and p27 in overexpressed WWOX cells ($\bar{x} \pm s$)

组别	细胞周期比例/%			蛋白表达水平	
	G ₀ /G ₁	S	G ₂ /M	Cyclin-D1	p27
Lenti NC	38.69 ± 4.15	51.63 ± 6.28	5.02 ± 0.41	0.76 ± 0.09	0.30 ± 0.04
Lenti WWOX	47.92 ± 2.47*	43.01 ± 4.15*	10.74 ± 2.23*	0.41 ± 0.03*	0.43 ± 0.05*
Lenti NC+FH535	48.10 ± 3.45*	41.89 ± 3.45*	10.01 ± 1.45*	0.37 ± 0.07*	0.45 ± 0.06*
Lenti WWOX+FH535	57.16 ± 2.06* [‡]	31.73 ± 2.06* [‡]	15.11 ± 1.82* [‡]	0.25 ± 0.03* [‡]	0.68 ± 0.08* [‡]
F	17.289	10.945	19.427	38.939	21.248
P	0.001	0.003	0.001	<0.001	<0.001

与Lenti NC相比, * $P < 0.05$; 与Lenti WWOX相比, * $P < 0.05$; 与Lenti NC+FH535比, [‡] $P < 0.05$ 。

Compared with Lenti NC, * $P < 0.05$; compared with Lenti WWOX, * $P < 0.05$; compared with Lenti NC+FH535, [‡] $P < 0.05$.

表5 Wnt信号通路抑制剂FH535对过表达WWOX的细胞凋亡率及细胞中C-Caspase-3蛋白表达影响($\bar{x} \pm s$)

Table 5 Effects of Wnt signal pathway inhibitor FH535 on apoptosis rate and C-Caspase-3 protein expression in overexpressed WWOX cells ($\bar{x} \pm s$)

组别	凋亡率/%	C-Caspase-3
Lenti NC	4.12 ± 0.36	0.08 ± 0.02
Lenti WWOX	18.63 ± 1.42*	0.16 ± 0.03*
Lenti NC+FH535	16.20 ± 1.68*	0.17 ± 0.02*
Lenti WWOX+FH535	28.46 ± 2.17* [‡]	0.26 ± 0.04* [‡]
F	124.182	19.727
P	0.000	0.001

与Lenti NC相比, * $P < 0.05$; 与Lenti WWOX相比, * $P < 0.05$; 与Lenti NC+FH535比, [‡] $P < 0.05$ 。

Compared with Lenti NC, * $P < 0.05$; compared with Lenti WWOX, * $P < 0.05$; compared with Lenti NC+FH535, [‡] $P < 0.05$.

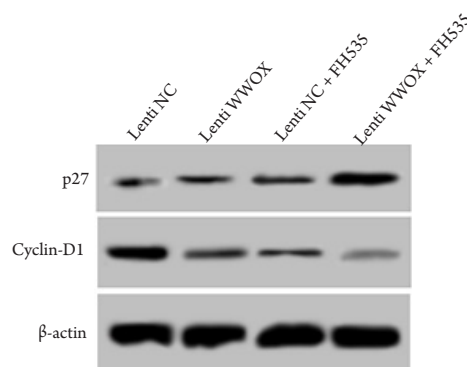


图3 Western印迹法检测Wnt信号通路抑制剂FH535对过表达WWOX的细胞中周期蛋白Cyclin-D1, p27表达影响

Figure 3 Western blot analysis of the effect of Wnt signaling pathway inhibitor FH535 on cyclin-D1 and p27 expression in WWOX overexpressed cells

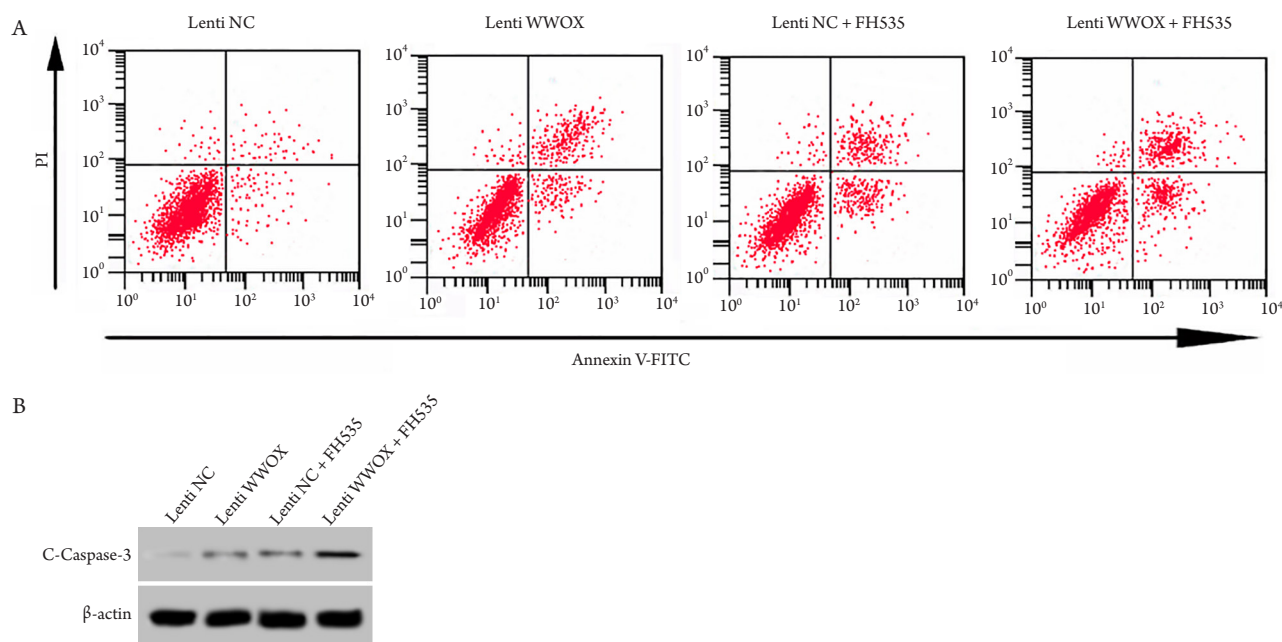


图4 Wnt信号通路抑制剂FHS35对过表达WWOX的细胞凋亡及凋亡蛋白C-Caspase-3表达影响

Figure 4 Effect of Wnt signaling pathway inhibitor FHS35 on apoptosis and expression of apoptotic protein C-Caspase-3 in WWOX-overexpressed cells

(A)流式细胞术检测细胞凋亡情况；(B)Western印迹法检测细胞中C-Caspase-3蛋白水平。

(A) Apoptosis was detected by flow cytometry; (B) Western blot was used to detect the level of C-Caspase-3 protein in cells.

3 讨论

WWOX基因位于16q23.3~24染色体上,其含有FRA16D脆性位点,其编码的蛋白质的N端含有2个WW结构域,C端含有短链脱氢还原酶,2个WW结构域之间有Caspase识别序列、线粒体靶向序列、底物结合位点,短链脱氢还原酶结构与SDR蛋白具有同源性^[7-8]。目前的研究^[9-12]显示:WWOX在肿瘤中发挥抑癌基因的作用,在肺癌、骨肉瘤、胆囊癌、卵巢癌等多种肿瘤中已经证实,WWOX具有调控肿瘤细胞生长作用,在肿瘤细胞恶性表型中发挥抑制功能。研究^[13]显示:WWOX在白血病患者中低表达,并且WWOX在白血病患者中甲基化水平高于正常对照组,WWOX甲基化可能是其表达减少或者缺失的原因,WWOX可能参与白血病细胞生长调控过程。本研究表明:过表达WWOX后的急性淋巴细胞白血病细胞的增殖能力降低,过表达WWOX发挥抗急性淋巴细胞白血病细胞作用。

细胞周期和细胞凋亡均为细胞正常生物学特性,在组织胚胎发育、器官生长、组织修复、疾病发生等过程中具有重要作用^[14]。细胞周期是细胞增殖的基础,受到细胞内多种因子的共同调

控作用,Cyclin-D1是细胞周期促进因子,其在G₀/G₁期表达升高,能够促进细胞从G₀/G₁期向S期转变,p27是与Cyclin-D1作用相反的周期调控因子,其在细胞周期进展中发挥抑制作用^[15-16]。Caspase-3是Caspase级联反应的下游执行因子,其活化后是细胞凋亡进入不可逆阶段的标志^[17]。目前在前列腺癌、膀胱癌、胰腺癌等肿瘤细胞中的研究^[18-20]显示:WWOX基因具有将细胞周期阻滞在G₀/G₁期的作用,并且能够抑制细胞中p27蛋白表达,促进细胞凋亡,诱导细胞中活化的Caspase-3蛋白表达。本研究表明:过表达WWOX后的急性淋巴细胞白血病细胞凋亡增多,细胞G₀/G₁期比例升高,细胞中Cyclin-D1蛋白水平降低,p27蛋白水平升高,活化的Caspase-3蛋白表达水平升高,过表达WWOX具有阻滞急性淋巴细胞白血病细胞周期并诱导凋亡作用。

Wnt在人体内具有多种生理学功能,与多种细胞的生长、分化、极性调控等有关,在胚胎轴向发育、神经生长等过程中扮演重要角色^[21]。Wnt/β-catenin是Wnt经典的信号通路,其中β-catenin是信号转导的关键蛋白,Wnt信号存在时,β-catenin不能被及时降解,过量的β-catenin转移至细胞核内,引起下游靶基因c-myc,Cyclin-D1等的表

达, Cyclin-D1是细胞从G₀/G₁期向S期转变的促进因子, Wnt/ β -catenin信号通路激活可以促进细胞周期进展, 诱导细胞增殖^[22-24]。目前研究显示: WWOX调控肿瘤细胞生长过程与Wnt/ β -catenin信号通路有关, WWOX表达下调可以诱导肝癌细胞中的 β -catenin积累, 激活Wnt/ β -catenin信号通路^[6,25,26]。本研究表明: 高表达WWOX能够降低急性淋巴细胞白血病细胞中 β -catenin的表达, 抑制Wnt/ β -catenin信号通路激活, 降低c-myc, Cyclin-D1表达水平, 并且Wnt/ β -catenin信号通路抑制剂可以协同WWOX共同抑制急性淋巴细胞白血病细胞增殖, 阻滞细胞周期, 诱导细胞凋亡, 说明高表达WWOX可以通过降低Wnt/ β -catenin信号通路激活水平发挥抗急性淋巴细胞白血病细胞增殖作用。

综上所述, WWOX能够阻滞急性淋巴细胞白血病细胞周期进展, 诱导细胞凋亡, 抑制细胞增殖, 其作用机制与降低Wnt/ β -catenin信号通路激活水平有关, 对于其具体的靶向作用位点还需要在以后的实验中进行探讨。本研究结果为明确WWOX在急性淋巴性白血病发生中的作用奠定了基础, 对研究急性淋巴白血病发病机制具有重要意义。

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本文引用: 罗剑锋, 罗丹, 文丹宁. 慢病毒介导的WWOX表达对急性淋巴细胞白血病细胞增殖活性的影响[J]. 临床与病理杂志, 2018, 38(11): 2321-2328. doi: 10.3978/j.issn.2095-6959.2018.11.006

Cite this article as: LUO Jianfeng, LUO Dan, WEN Danning. Effect of lentivirus mediated WWOX expression on proliferation of acute lymphoblastic leukemia cells[J]. Journal of Clinical and Pathological Research, 2018, 38(11): 2321-2328. doi: 10.3978/j.issn.2095-6959.2018.11.006