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七氟烷预处理对肺癌细胞凋亡与增殖的影响及机制分析*

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摘要 目的:探讨与分析七氟烷预处理对肺癌细胞凋亡与增殖的影响及机制。**方法:**采用不同浓度的七氟烷处理肺癌细胞系 A549, 采用噻唑蓝法检测细胞增殖抑制情况, 流式细胞术检测细胞凋亡, Transwell 实验检测细胞转移与侵袭, 采用免疫印迹法明确七氟烷的具体作用机制。**结果:**七氟烷处理可导致 A549 细胞增殖率显著降低, 细胞凋亡率显著增加($P<0.05$), 且存在剂量依赖性($P<0.05$)。同时细胞转移与侵袭指数、基质细胞衍生因子-1 (Stromal cell-derived factor 1, SDF-1)、CXC 类趋化因子受体 7 (CXC chemokine receptor 7, CXCR7) 蛋白相对表达量显著降低($P<0.05$), 也存在剂量依赖性($P<0.05$)。**结论:**七氟烷预处理能抑制肺癌细胞的 SDF-1/CXCR7 信号通路, 从而促进细胞凋亡, 抑制细胞增殖、转移与侵袭。

关键词:七氟烷; 肺癌; 细胞凋亡; 细胞增殖; SDF-1/CXCR7 信号通路

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Effects of Sevoflurane Preconditioning on Apoptosis and Proliferation of Lung Cancer Cells and Its Mechanism*

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ABSTRACT Objective: To investigate and analysis the effects of sevoflurane preconditioning on apoptosis and proliferation of lung cancer cells. **Methods:** The lung cancer cell line A549 were treated with different concentrations of sevoflurane. The inhibition of cell proliferation were detected by thiazolyl blue method. The apoptosis were detected by flow cytometry. The cell transfer and invasion were detected by Transwell assay. The sevoflurane were identified by immunoblotting. **Results:** The treatment of sevoflurane resulted in significant decreased in the proliferation rate of A549 cells ($P<0.05$), and were significant increased in apoptosis rate ($P<0.05$), and there were dose-dependent ($P<0.05$). At the same time, the relative expression of cell transfer and invasion index, Stromal cell-derived factor 1 (SDF-1) and CXC chemokine receptor 7 (CXCR7) protein were significantly decreased ($P<0.05$), and there were also dose-dependent ($P<0.05$). **Conclusion:** Sevoflurane preconditioning can inhibit the SDF-1/CXCR7 signaling pathway in lung cancer cells, thereby promoting apoptosis and inhibiting cell proliferation, metastasis and invasion.

Key words: Sevoflurane; Lung cancer; apoptosis; Cell proliferation; SDF-1/CXCR7 signal pathway

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前言

肺癌在我国发病率、死亡率逐年增加^[1-3]。手术为肺癌的主要治疗方法,但术后容易出现复发和疾病进展进而导致死亡,并且很多患者在就诊时已经为疾病晚期,失去了手术指征^[4,5]。有研究显示在肺癌发展中伴随有细胞极性形成、上皮细胞间黏附连接丧失,可增加其增殖,降低凋亡^[6-8]。当前的结果显示肿瘤凋亡与增殖与多种因素相关。SDF-1 是趋化因子的一员,与 CXCR7 具有高度的亲和力,在调控造血干细胞迁移有非常重要的作用^[9-11]。七氟烷是一种氟类吸入型麻醉药,具有过程平稳,诱导迅速、血气分配系数低、过程平稳麻醉深度容易调节等

特点^[12,13]。对于肺癌的治疗,无论何种术式,均需要麻醉,临床上常用于肺癌手术麻醉的药物为丙泊酚,但是部分患者会出现短暂性记忆失却、呼吸抑制或苏醒延迟等不良反应,也会诱发心血管意外,尤其是伴生理功能呈退行性改变的老年患者,可能会增加手术失败的风险^[14,15]。七氟烷麻醉也抑制改善单肺通气时的炎症反应,从而促进肿瘤细胞凋亡,抑制肿瘤增殖^[16,17],但是对肺癌细胞的影响尚无相关报道。本文具体探讨并分析了七氟烷预处理对肺癌细胞凋亡与增殖的影响及机制,以期七氟烷对于肺癌患者的安全应用提供实验依据。现报道如下。

1 材料与方法

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1.1 实验材料

主要细胞:肺癌细胞系 A549 由本实验室在液氮中保存,培养条件:含 10 %血清的 DMEM 培养液,5 % CO₂,37 °C 孵箱。取对数生长期细胞进行实验。

主要试剂:七氟烷购自上海恒瑞公司,兔抗 SDF-1 多克隆抗体(1:1000)与兔抗 CXCR7 多克隆抗体(1:1000)都购自碧云天生物技术研究所。

1.2 实验方法

(1)细胞分组与处理:将肺癌细胞分为三组,对照组进行常规培养。实验 1 组与实验 2 组将细胞培养箱入口通入七氟烷(浓度分别为 1 %与 3 %),都处理 2 h 后进行常规培养。(2)细胞增殖:将细胞接种于 96 孔板,每孔 200 μ L (1.5 $\times$ 10⁴ 个/mL 左右),首先每孔加 20 μ L 噻唑蓝,37°C 4 h,再加入 150 μ L 二甲基亚砷,避光 10 min,酶标法测定 490 nm 处的吸光度值。(3)细胞凋亡:采用孵育缓冲液洗涤细胞悬液 1 次,加入 100 μ L 异硫氰酸荧光素缓冲液,避光 10 min;弃上清,加入碘化丙啶,重悬

细胞,4 °C 避光 20 min,采用流式细胞术检测。(4)细胞转移与侵袭:细胞消化为单细胞悬液,取 200 μ L 垂直加入 Transwell 小室,在下室加入 500 μ L 含血清培养液。培养 24 h 后,固定小室,结晶紫溶液染色,计算细胞的转移与侵袭。(5)蛋白检测:细胞处理后收集细胞,加入 RIPA 裂解液提取总蛋白,蛋白定量后进行 SDS 聚丙烯酰胺凝胶电泳,免疫印迹法检测 SDF-1、CXCR7、 β -actin 表达情况。

1.3 统计学方法

应用 SPSS 22.0,计量资料采用($\bar{x}\pm s$)表示,行 t 检验, $P<0.05$ 有统计学意义。

2 结果

2.1 细胞增殖指数对比

与对照组相比,实验组的细胞增殖率显著降低($P<0.05$),且实验 2 组低于实验 1 组($P<0.05$)。见表 1。

表 1 三组细胞处理后不同时间点的增殖指数对比($\bar{x}\pm s$)

Table 1 Comparison of proliferation index at different time points after treatment of three groups of cells ($\bar{x}\pm s$)

Group	n	24 h	36 h
Control group	4	78.33 $\pm$ 2.93	89.33 $\pm$ 4.10
Experiment group 1	4	56.02 $\pm$ 4.44*	71.03 $\pm$ 5.19
Experimentgroup 2	4	45.00 $\pm$ 4.19**	54.09 $\pm$ 5.87#
F		12.843	15.305
P		0.000	0.000

Note: compared with the control group,* $P<0.05$; compared with the experimental group 1, # $P<0.05$.

2.2 细胞凋亡指数对比

与对照组相比,实验组的细胞凋亡率显著增加($P<0.05$),且

实验 2 组高于实验 1 组($P<0.05$)。见表 2。

表 2 三组细胞处理后不同时间点的凋亡指数对比($\bar{x}\pm s$)

Table 2 Comparison of apoptotic indices at different time points after treatment of three groups of cells ($\bar{x}\pm s$)

Group	n	24 h	36 h
Control group	4	4.44 $\pm$ 0.83	5.43 $\pm$ 0.22
Experiment group 1	4	14.81 $\pm$ 2.27*	15.31 $\pm$ 1.73*
Experimentgroup 2		18.92 $\pm$ 2.21**	20.14 $\pm$ 2.83**
F		25.024	21.942
P		0.000	0.000

2.3 细胞转移与侵袭指数对比

与对照组相比,实验组的细胞转移与侵袭指数显著降低

($P<0.05$),且实验 2 组低于实验 1 组($P<0.05$)。见表 3。

表 3 三组细胞处理后 36 h 的转移与侵袭指数对比($\bar{x}\pm s$)

Table 3 Comparison of metastasis and invasion index at 36 h after treatment of three groups of cells ($\bar{x}\pm s$)

Groups	n	Cell transfer index	Cell invasion index
Control group	4	33.14 $\pm$ 2.48	34.98 $\pm$ 3.11
Experiment group 1	4	17.45 $\pm$ 1.21*	18.33 $\pm$ 1.37*
Experimentgroup 2	4	8.83 $\pm$ 0.38**	9.22 $\pm$ 0.39**
F		13.092	10.944
P		0.000	0.001

2.4 蛋白表达情况对比

与对照组相比,实验组的 SDF-1、CXCR7 蛋白相对表达量

显著降低($P<0.05$),且实验 2 组低于实验 1 组($P<0.05$)。见表 4。

表 4 三组细胞处理后 36h 的 SDF-1、CXCR7 蛋白相对表达量对比($\bar{x}\pm s$)

Table 4 Comparison of relative expression levels of SDF-1 and CXCR7 proteins at 36 h after treatment with three groups of cells ($\bar{x}\pm s$)

Groups	n	SDF-1	CXCR7
Control group	4	4.86± 0.33	4.16± 0.10
Experiment group 1	4	2.83± 0.44*	2.14± 0.08*
Experimentgroup 2	4	1.22± 0.03*#	1.27± 0.12*#
F		9.824	8.772
P		0.001	0.003

3 讨论

当前随着我国城市化进程的加快,肺癌的发病率显著升高^[18-20]。肺癌是我国乃至全世界最常见的恶性肿瘤之一,其发病率不断上升,非小细胞肺癌为肺癌的主要类型。七氟烷为无色透明、无刺激性的挥发性液体,麻醉诱导快、麻醉维持平稳、苏醒快,被广泛用于恶性肿瘤的手术过程中^[21-23]。而吸入性麻醉药可对麻醉本身产生影响,也会对肿瘤细胞的生物学行为产生影响^[24,25]。本研究显示与对照组相比,实验组的细胞增殖率、细胞转移与侵袭指数显著降低,且实验 2 组低于实验 1 组,表明七氟烷的应用能抑制细胞增殖、转移与侵袭,与当前也有研究类似^[26-28],发现七氟烷能显著抑制 A549 细胞增殖并呈时间与剂量依赖性,特别是随着七氟烷浓度的增加,A549 细胞也逐渐发生周期紊乱,可下调细胞周期抑制细胞增殖。推测可能原因是改善单肺通气时的炎症细胞反应,从而促进肿瘤细胞凋亡,抑制肿瘤增殖。

本研究显示细胞处理后 24 h 与 36 h,与对照组相比,实验组的细胞凋亡率显著增加,且实验 2 组高于实验 1 组,表明七氟烷能促进细胞凋亡,使机体清除无用的细胞,与 Zhu Y 的研究 MicroRNA-613 抑制肺转移类似,说明抑制凋亡能促进肿瘤性转化,影响对抗癌治疗^[29]。提示七氟烷预处理可减少肺组织细胞凋亡,抑制中性粒细胞向内皮细胞的黏附,减少炎性介质的释放,减轻肺组织损伤,抑制肺组织细胞的凋亡^[30]。

SDF-1/CXCR7 生物学轴可参与非小细胞肺癌的发生发展,能够促进肠上皮细胞 NF- κ 的激活,可通过减少 E- 钙黏蛋白来达到促进粘膜修复的作用。SDF-1/CXCR7 生物学轴在结直肠癌、肝癌、结直肠癌、食管癌中均呈现低表达状态,肿瘤细胞的高侵袭转移能力与 SDF-1/CXCR7 生物学轴激活显著相关。本研究实验组的 SDF-1、CXCR7 蛋白相对表达量显著低于对照组,且实验 2 组低于实验 1 组,表明七氟烷的应用能抑制 SDF-1/CXCR7 信号通路,从而发挥抑癌作用^[31]。提示七氟烷也能够保护肺脏,可通过抑制其下游基因的表达从而抑制缺氧微环境中肺癌细胞增殖^[32-34]。本研究也有一定的不足之处,七氟烷对 SDF-1/CXCR7 的具体机制尚不清楚,且研究组别分组还不够细致,且没有对照的麻醉药物,有待进一步研究。

综上,七氟烷预处理能抑制肺癌细胞的 SDF-1/CXCR7 信号通路,从而促进细胞凋亡,抑制细胞增殖、转移与侵袭。

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