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· 实验研究 ·

3T3 细胞源外泌小体对小鼠结直肠癌细胞 CT26 增殖的影响

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【摘要】 **目的** 观察小鼠成纤维细胞株 3T3 来源的外泌小体(exosome)对小鼠结直肠癌细胞 CT26 增殖能力的影响,并探讨其机制。**方法** 通过 PureExo exosome 提取试剂盒提取 3T3 细胞培养基上清中的 exosome,按照不同浓度及时间作用于 CT26 细胞,并利用细胞计数试剂盒(CCK-8)检测 CT26 细胞增殖能力,碘化丙锭(PI)染色法检测细胞周期改变,Western blot 及实时定量反转录聚合酶链反应(RT-qPCR)检测 CT26 中 DNA 甲基转移酶 1(DNMT1)及抑癌基因 p16 的蛋白及 mRNA 表达。**结果** CCK-8 实验中 450 nm 处吸光度(A)值 96 h 时,exosome 处理组(10 mg/L:1.17 ± 0.04,50 mg/L:1.69 ± 0.03,200 mg/L:2.08 ± 0.05),显著高于对照组(Control):0.89 ± 0.03,即随着 exosome 处理浓度的提高,CT26 细胞增殖逐渐增快;同时细胞周期进程加快,表现为处于 S 期及 G_2/M 期的细胞比例增多,处于 G_0/G_1 期比例减少。而 Western blot 提示调控 DNA 甲基化的重要蛋白 DNMT1 明显增多,同时伴有其下游蛋白及细胞增殖、周期相关的分子如 p16、p21、细胞周期素(Cyclin) D1 和 CDK6 的水平变化,RT-qPCR 也验证了上述分子 mRNA 水平的变化。**结论** 小鼠成纤维细胞株 3T3 来源 exosome 可促进小鼠结直肠癌细胞 CT26 增殖,加快其细胞周期进程,并且可能通过 DNMT1 改变其下游分子发挥上述作用。

【关键词】 外泌小体; 结直肠癌; 增殖; 细胞周期; DNA 甲基转移酶 1

Effect of exosome extracted from 3T3 on proliferation of mouse colorectal cancer cells CT26 *Zhu Dongliang**, *Liu Zhisu*, *Wang Fangyuan*. **Department of General Surgery, South Central Hospital of Wuhan University, Wuhan 430071, China*

【**Abstract**】 **Objective** To investigate the effect of exosome secreted by 3T3 on proliferation of mouse colorectal cancer cells CT26, and explore the potential underlying mechanism. **Methods** The exosome was obtained and purified by PureExo exosome kit from mouse fibroblast 3T3 cells cultural supernatant, and CT26 cells were cultured and incubated with different doses of exosome for indicated time. Then cell counting kit-8 (CCK-8) assay and propidium iodide (PI) incorporation were performed to measure the cell proliferation and cell cycle of CT26. Western blotting and real-time quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR) were used to detect and analyze the potential mechanism. **Results** The CCK-8 assay showed at A 450 nm, 96 h, absorption of exosome group (10 mg/L: 1.17 ± 0.04, 50 mg/L: 1.69 ± 0.03, 200 mg/L: 2.08 ± 0.05), were obvious higher than Control group (0.89 ± 0.03). The CT26 cells treated by exosome had faster proliferation and more aggressive cell cycle compared with untreated cells in Control group. Meantime, exosome also increased the expression of DNA methyltransferase 1 (DNMT1), with elevating the expression of p16 and p21, and decreasing the expression of cyclin D1 and CDK6, which were observed by Western blotting and qPCR. **Conclusion** The exosome extracted from mouse fibroblast 3T3 cells could promote the proliferation and cell cycle progress of mouse colorectal cancer cells CT26, and DNMT1 might play an important role in this regulation.

【**Key words**】 Exosome; Colorectal cancer; Proliferation; Cell cycle; DNA methyltransferase 1

近年来研究结果显示,肿瘤微环境与肿瘤的疾病进程及预后密切相关,肿瘤内或肿瘤周围的炎性细胞、间质细胞可通过分泌细胞因子等多种方式调节肿瘤细胞的增殖、分化、侵袭转移及血管生成等一系列病理生理功能[1-2]。其中外泌小体(exosome)作为近年来研究的热点,被认为在肿瘤的发生发展中起重要作用。exosome 也称为小囊体或小囊泡,直径为 30 ~ 100 nm,是细胞外泌囊泡中体积较小的一种,其中包含多种蛋白及 RNA 分子。研究表明,间质细胞可以通过分泌 exosome 激活乳腺癌细胞中的转录信号转导子与激活子 1(STAT1)及 Notch 信号通路,调节肿瘤的化疗敏感性;exosome 也有望作为非小细胞肺癌新的治疗靶点及临床诊断标志[3-4]。我们通过小鼠成纤维细胞株 3T3

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