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mTOR抑制剂FIM-A对人骨肉瘤细胞株MG-63的抑制作用及其机制 点此下载全文

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摘要：

目的： 观察新型哺乳动物雷帕霉素靶蛋白（mammalia target of rapamycin，mTOR）抑制剂含磷西罗莫司衍生物FIM-A对人骨肉瘤MG-63细胞增殖及凋亡的影响。 方法： 不同浓度（1×10-9~1×10-5 mol/L） FIM-A处理MG-63细胞后，采用CCK-8法检测MG-63细胞的增殖，流式细胞术检测MG-63细胞周期和凋亡情况，ELISA法检测血管内皮细胞生长因子（vascular endothelial cell growth factor，VEGF）和低氧诱导因子（hypoxia inducible factor-1α，HIF-1α）的分泌量，RT-PCR和Western blotting分别检测FIM-A对MG-63细胞中 mTOR 、p70核糖体S6激酶（p70S6 kinase protein，p70s6k）及4E结合蛋白1（4E-binding protein 1， 4E-BP1 ）mRNA和蛋白表达的影响。 结果： 与人成骨hF-OB1.19细胞相比，人骨肉瘤MG-63细胞中mTOR、p70s6k及4E-BP1 mRNA的表达水平明显升高（ P <0.05）。FIM-A可有效抑制MG-63细胞的增殖（ P <0.05），且呈剂量依赖性（ r =0 940， P <0.01）。1×10-6 mol/L FIM-A 处理24 h后与对照组相比，G 0/G 1期MG-63细胞比例明显增加\[（56.4±3.2）% vs （43.4±6.9）%， P <0.05\]，而MG-63细胞的凋亡率没有明显改变。不同浓度FIM-A作用24 h后，MG-63细胞中HIF-1α和VEGF表达均明显低于对照组（ P <0.05），且具有剂量依赖性（HIF-1α， r =-0.988， P <0.01；VEGF， r =-0.998， P <0.01）。同时，FIM-A对MG-63细胞中mTOR（ r=- 0.919， P <0.01）、p70s6k（ r=- 0.843， P <0.01）及4EBP1（ r=- 0.818， P <0.01）蛋白的磷酸化也具有浓度依赖性抑制作用。 结论： FIM-A能抑制人骨肉瘤MG-63细胞的增殖，并阻滞细胞周期于G 0/G 1期，其机制可能与影响mTOR信号通路蛋白磷酸化有关。

关键词：mTOR抑制剂 FIM-A 骨肉瘤 MG-63细胞 mTOR

Inhibitory effect of FIM-A, a mTOR inhibitor, on the proliferation and apoptosis of human MG-63 osteosarcoma cell line and its mechanism Download Fulltext

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Abstract:

Objective: To investigate the effect of phosphorus sirolimus derivatives FIM-A, a new mammalian mammalia target of rapamycin (mTOR) inhibitor, on the proliferation and apoptosis of human MG-63 osteosarcoma cell line. Methods: Human MG-63 osteosarcoma cells and hF-OB1.19 osteoblasts were cultured in vitro and incubated with different concentrations of FIM-A (1×10-9-1×10-5 mol/L) for 24 hours. CCK-8 assay was used to evaluate the cell proliferation. The cell cycle and apoptosis were analyzed using flow cytometry. ELISA was used to detect the secretions of vascular endothelial cell growth factor (VEGF) and hypoxia inducible factor-1α (HIF-1α). The expressions of mTOR, p70S6 kinase protein (p70s6k) and 4E-binding protein 1 (4E-BP1) mRNA and protein were detected by RT-PCR and Western blotting, respectively. Results: The expressions of mTOR, p70s6k and 4E-BP1 mRNA in MG-63 osteosarcoma cells were significantly higher than that in the hF-OB1.19 osteoblasts (P <0.05). The proliferation of the MG-63 osteosarcoma cells were significantly inhibited after FIM-A treatment. The proliferation inhibition rate of MG-63 cells was significantly higher than that of the negative control group after the treatment of 1×10-7 mol/L FIM-A (\[37.64±2.07\]% vs 0, P <0.05), and the cell proliferation inhibition rate increased along with FIM-A concentrations in a dose-dependent manner (r =0 940, P <0.01). After the treatment of 1×10-6 mol/L FIM-A for 24 hours, the proportion of MG-63 cells in G 0/G 1 phase was significantly increased compared with the control group (\[56.4±3.2\]% vs \[43.4±6.9\]%, P <0 05). No obvious changes were found in the apoptotic rate of MG-63 cells compared with the control group. The expression levels of HIF-1 α and VEGF in MG-63 cells were significantly lower than those of the control group after the treatment of different concentrations of FIM-A for 24 hours (P <0 05), and as concentrations increased, the level of HIF-1 α (r=0.988, P <0 01) and VEGF (r=0.998, P <0.01) decreased gradually in a dose-dependent manner. Meanwhile, the inhibitory effect of FIM-A on phosphorylations of p-mTOR (r=- 0.919, P <0.01), p-p70s6k (r=- 0.843, P <0.01) and p-4EBP1 (r=- 0.818, P <0.01) proteins in MG-63 cells was also in a dose-dependent manner. Conclusion: FIM-A can significantly inhibit human MG-63 osteosarcoma cells and induce G 0/G 1 phase cell cycle arrest, and its anti-tumor effect was probably through the intervention of mTOR pathway.

Keywords:mTOR inhibitor FIM-A osteosarcoma MG-63 cell mTOR

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