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基础研究

RAIL和endostatin双基因-放射治疗对人血管内皮细胞增殖、周期和凋亡的影响

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摘要：

目的：观察重组质粒pshuttle-Egr1-shTRAIL-shES携带的双基因TRAIL和endostatin联合X射线照射后，对血管内皮细胞ECV304增殖、周期和凋亡的影响。方法：实验分为对照组、空载体pshuttle转染组、TRAIL单基因重组质粒pshuttle-Egr1-shTRAIL转染组、endostatin单基因重组质粒pshuttle-Egr1-shES转染组和TRAIL、endostatin双基因重组质粒pshuttle-Egr1-shTRAIL-shES转染组。细胞转染采用脂质体介导的方法进行，对照组不转染。细胞转染后给予X射线照射（照射剂量分别为0、0.1、0.5、1.0、2.0和5.0 Gy），采用ELISA法检测转染细胞中TRAIL和endostatin蛋白的表达，并分别采用MTT及PI单染或/和Annexin Ⅴ双染流式细胞术（FCM）检测TRAIL、endostatin单/双基因治疗联合放射治疗对ECV304细胞增殖、细胞周期和凋亡的影响。结果：2.0 Gy X射线照射后与0 h比较，各时间点转染pshuttle-Egr1-shTRAIL-shES的ECV304细胞上清中TRAIL和endostatin蛋白表达水平明显升高（P<0.01），分别于12和24 h达峰值；不同剂量X射线照射可诱导TRAIL和endostatin蛋白表达，且蛋白表达水平随照射剂量的增加而明显升高（P<0.05或P<0.01）。MTT结果显示，X射线照射后， pshuttle-Egr1-shTRAIL、pshuttle-Egr1-shES和pshuttle-Egr1-shTRAIL-shES组ECV304细胞A 490值均明显低于对照组和pshuttle组，并显示一定的时间-效应和剂量-效应关系，并伴有细胞凋亡率明显增加、G_2+M期细胞百分数明显上升和G_0/G_1期细胞百分数明显下降。上述细胞效应，尤以pshuttle-Egr1-shTRAIL-shES组变化最为明显，与pshuttle-Egr1-shTRAIL和pshuttle-Egr1-shES组比较差异有统计学意义（P<0.05 或 P<0.01）。结论： TRAIL和endostatin双基因联合放射治疗可抑制ECV304细胞生长，影响细胞周期进程，促进细胞凋亡，且其治疗效果优于单纯放射治疗或TRAIL/endostatin单基因-放射治疗。

关键词： 肿瘤坏死因子相关凋亡诱导配体；内皮抑素；早期生长反应-1启动子；基因-放射治疗

Effects of TRAIL and endostatin double-gene-radiotherapy on proliferation,cell cycle procession and apoptosis |in human vascular endothelial |cells

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Abstract:

Abstract:Objective
To study the effects of TRAIL and endostatin double genes in recombinant plasmid pshuttle-Egr1-shTRAIL-shES combined with X-ray irradiation on the proliferation,cell cycle procession and apoptosis in human vascular endothelial ECV304 cells.Methods Cells were divided into control,empty vector pshuttle,TRAIL single-gene plasmid pshuttle-Egr1-shTRAIL,endostatin single-gene plasmid pshuttle-Egr1-shES and TRAIL plus endostatin double-gene plasmid pshuttle-Egr1-shTRAIL-shES transfection groups.The cell transfection was done by lipofectamine-mediated method,and no transfection in control group.After plasmid transfection,the cells were exposed under X-ray irradiation at the doses of 0,0.1,0.5,1.0,2.0 and 5.0 Gy, respectively.Then the time-course and dose-effect patterns of the TRAIL and endostatin protein expressions induced by irradiation were detected by ELISA,and the effects of TRAIL and/or endostatin single or double gene therapy combined with radiotherapy on the proliferation,cell cycle procession and apoptosis in ECV304 cells were detected by MTT assay,flow cytometry (FCM) with PI single-staining or/and Annexin Ⅴ double-staining.Results After 2.0 Gy X-ray irradiation,the expression levels of TRAIL and endostatin proteins in the supernatant of cultured ECV304 cells transfected with pshuttle-Egr1-shTRAIL-shES at other time-points were increased when compared with those at 0 h (P<0.01),and reached to the peak value at 24 and 12 h, respectively.Furthermore,their expression levels of TRAIL and endostatin protein induced by irradiation were increased significantly with the enlargement of radiation doses (P<0.05 or P<0.01).Under the exposure of X-ray irradiation,as compared with those in control and pshuttle groups,the A 490 values detected with MTT in pshuttle-Egr1-shTRAIL,pshuttle-Egr1-shES and pshuttle-Egr1-shTRAIL-shES groups were reduced in a time- and dose-dependent manner,and the apoptotic rates and the cell percentages in G_2 + M phase were increased,while those in G_0/G_1 phase were declined.In particular,the cellular effects mentioned above in pshuttle-Egr1-shTRAIL-shES group were significantly higher than those in pshuttle-Egr1-shTRAIL and pshuttle-Egr1-shES groups(P<0.05 or P<0.01).Conclusion TRAIL and endostatin double-gene-therapy in combination with radiotherapy could inhibit cell proliferation,affect cell cycle procession and promote apoptosis in ECV304 cells,and its therapeutic efficacy is better than those of single gene-radiotherapy or radiotherapy.

Keywords: tumor necrosis factor related apoptosis inducing ligand endostatin early growth response-1 promoter gene-radiotherapy

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