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论著

端粒酶抑制剂叠氮胸苷对HeLa细胞放射性DNA损伤修复的影响

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摘要 背景与目的： 研究端粒酶抑制剂叠氮胸苷(Azidothymidine，AZT)对人宫颈癌HeLa细胞 DNA放射性损伤修复的影响，探讨端粒酶在放射诱导的DNA损伤修复中的作用。 材料与方法： 实验分为空白组，AZT组(400 μmol/L AZT处理HeLa细胞24 h)，放射组(2 Gy 60Co γ射线照射)，AZT放疗组(400 μmol/L AZT处理HeLa细胞24 h后，用2 Gy 60Co γ射线照射)。照射后于0、5、10、30、60、180及360 min分别收集细胞，用端粒重复序列扩增法(PCR_based telomeric repeat amplification protocol，TRAP)_联合酶联免疫吸附法(enzyme linked immunosorbent assay,ELISA)即TRAP_ELISA法检测端粒酶的活性。用单细胞凝胶电泳法检测DNA单链断裂损伤，以彗尾DNA百分含量表示DNA单链断损伤量。 结果： HeLa细胞受2 Gy 60Co γ射线照射后10 min，端粒酶活性即开始增加，60 min后增加明显，360 min时达到最高。AZT处理HeLa细胞后，能使端粒酶活性下降约50%，而且能抑制HeLa细胞照射后端粒酶活性的增加 (P<0.05)。单细胞凝胶电泳实验表明，2 Gy 60Co γ射线照射HeLa细胞后0～10 min，AZT放疗组与放射组的彗尾DNA百分含量无明显差异(P>0.05)，照后30～360 min AZT放疗组彗尾DNA百分含量均高于放射组(P均<0.05)。 结论： AZT能阻抑照射后30～ 360 min DNA单链断裂的修复，说明端粒酶可能在放射性DNA损伤修复中具有重要作用。

关键词 叠氮胸苷； HeLa细胞； 端粒酶； DNA修复

Effect of Telomerase Inhibitor Azidothymidine on Repair of DNA Damage Induced by Radiation in Human HeLa cells

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Abstract BACKGROUND & AIM: There are now many reports that telomerase activity can be upregulated in some cells following exposure to low doses radiation, and that the enzyme may be involved in the repair of radiation damage. We studied the effect of telomerase inhibitor Azidothymidine (AZT) on repair of DNA damage induced by irradiation in human HeLa cells. MATERIALS AND METHODS: There were four groups：control group, azidothymidine group (HeLa cells were pretreated by 400 μmol/L Azidothymidine for 24 h),radiation group(HeLa cells were irradiated with 2 Gy 60Co γ rays), azidothymidine/radiation group(HeLa cells were irradiated with 2 Gy 60Co γ rays,with pretreatment by 400 μmol/L Azidothymidine for 24 h). Telomerase activity was measured by a PCR_based telomeric repeat amplification protocol (TRAP) coupled with ELISA and DNA single_stranded breaks was evaluated by single cell gel electrophoresis assay (SCGE). RESULTS: Telomerase activity of HeLa cells began increasing at 10 minutes,more at 60 min,and peaked at 360 min after irradiation with 2 Gy 60Co γ rays,and decreased about 50% after pretreatment with Azidothymidine. Azidothymidine could inhibit telomerase activity increase after irradiation (P<0.05).In SCGE tests,0－10 min after irradiation with 2 Gy 60Co γ rays, there was no difference in percentage of DNA in the tail between Radiation group and Azidothymidine /Radiation group(P>0.05), but percentage of DNA in the tail of Azidothymidine /Radiation group was more than Radiation group(P<0.05)30－60 min after irradiation. CONCLUSION: Azidothymidine could reduce the repair of single_stranded DNA breaks 30－360 min after irradiation. These results suggested that telomerase could play an important role in irradiation_induced DNA damage repair.

Keywords azidothymidine HeLa cells telomerase DNA repair

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