

γ 射线照射 C6、SGH-446 与 NCI-H446 肿瘤细胞的 三维结构变化和 DNA 辐射损伤

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脑胶质瘤是常见恶性肿瘤,我国脑胶质瘤发病率占脑肿瘤 40% 以上,占全部肿瘤的 1%—3%,由于血-脑屏障作用,神经胶质瘤对射线并不敏感,放疗的治疗比值较小,化疗也不理想。寻找神经胶质瘤细胞的耐辐射基因,探讨其耐辐射机制,可望为脑胶质瘤放疗增敏提供指导,提高患者术后的生存率。本研究用小鼠脑胶质瘤(C6)、人脑胶质瘤(SHG-44)和小细胞肺癌(NCI-H446)等细胞株、用单细胞凝胶电泳技术和激光共聚焦扫描显微镜(Laser scanning confocal microscopy, LSCM),观察 γ 射线照射细胞的三维图象,研究 γ 射线所致DNA链断裂及脱氧核糖核酸与核糖核酸的比值(Deoxyribonucleic acid / ribonucleic acid, DNA/RNA)含量比值的变化。

C6、SHG-44、NCI-H446等细胞株购自中国科学院上海细胞研究所,荧光染料(Acridine orange, AO)为Sigma公司产品。LSCM, TCS-SP2型,Leica公司。用单细胞凝胶电泳技术在荧光显微镜下观察DNA链断裂迁移的距离。用激光共聚焦光束的针孔点光源扫描不同剂量照射的肿瘤细胞内焦平面,CT成象,观察DNA分子损伤的变化,并计算肿瘤细胞DNA/RNA含量比值,评价它们的损伤程度。实验结果如下:

(1) 以1、3、8Gy照射C6、SHG-44、NCI-H446细胞株,对照射组与照射组作细胞染色处理,所有样品均在观察前染色,用LSCM检测DNA/RNA含量变化。AO染料与DNA结合发出黄绿色和绿色荧光,与RNA结合发出橙色和红色荧光。结果发现,三种细胞对照组细胞内DNA荧光染色明显高于RNA荧光染色($p<0.01$),1、3Gy照射组DNA/RNA比值与对照组相比, $p<0.01$;8Gy与对照组相比, $p>0.05$ 。

(2) 各剂量点的NCI-H446细胞株的DNA损伤有明显差异,损伤程度随照射剂量的增加而加重,剂量-效应关系良好,而接受相同辐照剂量的脑胶质瘤细胞的辐射损伤程度较轻。

由此可见,三种肿瘤细胞的DNA辐射损伤有很大差别。LSCM观察到受照后细胞内DNA明显聚集,脑胶质瘤细胞DNA损伤程度与小细胞肺癌比较有显著差异($p<0.01$)。

关键词 共聚焦显微镜,细胞株(C6、SHG-44),脱氧核糖核酸,核糖核酸, ^{60}Co γ 辐照中图分类号 R811.5

3D structural changes and DNA damages to γ ray irradiated C6, SHG-44 and NCI-H446 tumor cells

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Because of its low sensitivity to radiations, therapeutic ratio of neuroglioma is relatively small. And chemotherapy is not ideal to the disease, either. Therefore, studies are needed towards finding radio-resistance gene.

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Human cerebral glioma SHG-44, mouse cerebral glioma C6, and small cell lung cancer NCI-H446 (for comparison), were irradiated by ^{60}Co γ -rays to 1, 3 and 8 Gy, and changes of DNA/RNA ratios in the cells were studied with LSCM (laser scanning confocal microscopy). It was found that the fluorescence dye of DNA in three kinds of cells in the control group is significantly higher than the RNA fluorescence dye ($p < 0.01$). Degree of the damages to the irradiated NCI-H446 cells increased gradually with the dose, and damages to the cerebral glioma cells was less severe at the same radiation dose as the NCI-H446 cells.

KEYWORDS Confocal microscope, Cell strains (NCI-H446, C6, SHG-44), Deoxyribonucleic acid (DNA), Ribonucleic acid (RNA), ^{60}Co γ -irradiation

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